

69861 U.S. PTO
09/25/97

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EM354411625US

PATENT
Atty. Docket No. CRP-070FWCN2
(2054/39)

65705 U.S. PTO
08/937756
09/25/97

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT(S): Rueger, et al.
SERIAL NO.: -----
FILED: Herewith
TITLE: Morphogen-Induced Nerve Regeneration and Repair
GROUP NO.: 1812
(Prior Application)
EXAMINER: R. Hayes
(Prior Application)

CERTIFICATE OF EXPRESS MAILING UNDER 37 C.F.R. 1.10

I hereby certify that the attached document is being deposited with the United States Postal Service, postage prepaid, on September 25, 1997 utilizing the "Express Mail Post Office to Addressee" service of the United States Postal Service, mailing label number EM354411625US, in an envelope addressed to: ATTN: BOX PATENT APPLICATION, Assistant Commissioner for Patents, Washington, DC 20231.

Brenda L. MacLean
Brenda L. MacLean

Box Patent Application
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

TRANSMITTAL OF FILING UNDER 37 CFR 1.60(b)

This is a request for filing a Continuation of application under 37 CFR 1.60, of pending prior application serial no. 08/ 260,675 filed on June 16, 1997 of Rueger, et al., for Morphogen-Induced Nerve Regeneration and Repair.

1. Copy of Prior Application as Filed Which is Attached

- ☒ I hereby verify that the attached papers are a true copy of what is shown in my records to be the above identified prior application, including the oath or declaration originally filed (37 CFR 1.60).

The copy of the papers of prior application as filed which are attached are as follows:

Transmittal Of Filing Under 37 CFR 1.60 (b)**Page 2**

147 page(s) of specification
11 page(s) of claims
1 page(s) of abstract
5 sheet(s) of drawing

2. Amendments

- ☒ Cancel in this application original claims 2-81 of the prior application before calculating the filing fee. (At least one original independent claim must be retained for filing purposes.)
- ☒ A preliminary amendment is enclosed. (Claims added by this amendment have been properly numbered consecutively beginning with the number next following the highest numbered **original** claim in the prior application.)

3. Fee Calculation (37 CFR 1.16)

CLAIMS AS FILED					
	Number Filed	Number Extra		Rate	Basic Fee 37 CFR 1.16(a) \$770.00
Total Claims (37 CFR 1.16 (c))	57	- 20 = 37	X	\$ 22.00	\$ 814.00
Independent Claims (37 CFR 1.16 (b))	18	- 3 = 15	X	\$ 80.00	\$ 1200.00
Multiple Dependent Claim(s), If any (37 CFR 1.16(d))	Yes		+	\$ 260.00	\$ 260.00

- ☐ Fee for extra claims is not being paid at this time. (37 CFR 1.16(d))

Filing Fee Calculation \$ 3,044.00

4. Small Entity Status

- ☒ A verified statement that this filing is by a small entity:
- ☐ is attached
- ☒ has been filed in the parent application and such status is still proper and desired (37 CFR 1.28(a))

Filing Fee Calculation (50% of above) \$ 1,522.00

5. Drawings

☐ Transfer the drawings from the prior application to this application and, subject to item 17 below, abandon said prior application as of the filing date accorded this application.

☒ Drawings are enclosed

☐ formal
☒ informal

6. Assignment

☒ The prior application is assigned of record to Creative BioMolecules, Inc. at Reel No. 6531 and Frame No. 0897.

☐ an assignment of the invention to _____

☐ is attached.

A separate "ASSIGNMENT COVER LETTER ACCOMPANYING NEW PATENT APPLICATION" is also attached.

NOTE: "If an assignment is submitted with a new application, send two separate letters - one for the application and one for the assignment." Notice of May 4, 1990 (1114 O.G. 77-78).

7. Fee Payment Being Made At This Time

☒ Enclosed

☒ basic filing fee \$ 1,522.00

8. Method of Payment of Fees

☒ enclosed is a check in the amount of \$ 1,522.00

☐ charge Account No. 20-0531 in the amount of \$ _____

A duplicate of this request is attached.

9. Authorization To Charge Additional Fees

☒ The Commissioner is hereby authorized to charge any deficiencies in fees to Account No. 20-0531.

Transmittal Of Filing Under 37 CFR 1.60 (b)

Page 4

☒ Address all future communications to:

Patent Administrator
Testa, Hurwitz & Thibault, LLP
High Street Tower
125 High Street
Boston, MA 02110

Dated: September 25, 1997

Reg. No. 36,989

Tel. No. (617) 248-7013



Thomas C. Meyers
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PATENT
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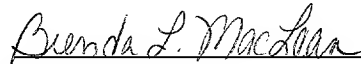


IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS: Rueger, *et al.*
SERIAL NUMBER: Continuation of 08/260,675
FILING DATE: Herewith
TITLE: Morphogen-Induced Nerve Regeneration and Repair
GROUP: 1812
(Prior Application)
EXAMINER: R. Hayes
(Prior Application)

CERTIFICATE OF MAILING UNDER 37 C.F.R. 1.10

I hereby certify that this correspondence, together with any documents referred to as being attached hereto is/are being deposited with the United States Postal Service as Express Mail, Mailing Label No. EM354411625US, postage prepaid, in an envelope addressed to: Box Patent Application, Assistant Commissioner for Patents, Washington D.C., 20231 on September 25, 1997.


Brenda L. MacLean

PRELIMINARY AMENDMENT

Box Patent Application
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

This amendment is submitted concurrently with the filing of a continuation application for the above referenced patent application. Consideration of the application in view of the amendments made herein respectfully is requested.

In the Claims:

Kindly cancel claim 1 and insert new claims 82-104 as follows:

--82. A method of treating amyotrophic lateral sclerosis, comprising administering a morphogen comprising a dimeric protein having an amino acid sequence selected from the group consisting of a sequence:

- (a) having at least 70% homology with the C-terminal seven-cysteine skeleton of human OP-1, residues 330-431 of SEQ ID NO: 2;
- (b) having greater than 60% amino acid sequence identity with said C-terminal seven-cysteine skeleton of human OP-1;
- (c) defined by Generic Sequence 7, SEQ ID NO: 4;
- (d) defined by Generic Sequence 8, SEQ ID NO: 5;
- (e) defined by Generic Sequence 9, SEQ ID NO: 6;
- (f) defined by Generic Sequence 10, SEQ ID NO: 7, and
- (g) defined by OPX, SEQ ID NO: 3,

wherein said morphogen stimulates production of an N-CAM or L1 isoform by an NG108-15 cell *in vitro*.

83. A method of treating multiple sclerosis, comprising administering a morphogen comprising a dimeric protein having an amino acid sequence selected from the group consisting of a sequence:

- (a) having at least 70% homology with the C-terminal seven-cysteine skeleton of human OP-1, residues 330-431 of SEQ ID NO: 2;
- (b) having greater than 60% amino acid sequence identity with said C-terminal seven-cysteine skeleton of human OP-1;
- (c) defined by Generic Sequence 7, SEQ ID NO: 4;
- (d) defined by Generic Sequence 8, SEQ ID NO: 5;
- (e) defined by Generic Sequence 9, SEQ ID NO: 6;
- (f) defined by Generic Sequence 10, SEQ ID NO: 7, and
- (g) defined by OPX, SEQ ID NO: 3,

wherein said morphogen stimulates production of an N-CAM or L1 isoform by an NG108-15 cell *in vitro*.

84. A method of treating a spinal cord injury, comprising administering a morphogen comprising a dimeric protein having an amino acid sequence selected from the group consisting of a sequence:

- (a) having at least 70% homology with the C-terminal seven-cysteine skeleton of human OP-1, residues 330-431 of SEQ ID NO: 2;
- (b) having greater than 60% amino acid sequence identity with said C-terminal seven-cysteine skeleton of human OP-1;
- (c) defined by Generic Sequence 7, SEQ ID NO: 4;
- (d) defined by Generic Sequence 8, SEQ ID NO: 5;
- (e) defined by Generic Sequence 9, SEQ ID NO: 6;
- (f) defined by Generic Sequence 10, SEQ ID NO: 7, and
- (g) defined by OPX, SEQ ID NO: 3,

wherein said morphogen stimulates production of an N-CAM or L1 isoform by an NG108-15 cell *in vitro*.

85. The method of claim 84, wherein said spinal cord injury results from a mechanical trauma.
86. The method of claim 84, wherein said spinal cord injury results from a tumor.
87. The method of claim 84, wherein said spinal cord injury results from a chemical trauma.
88. A method of restoring motor function in a mammal afflicted with amyotrophic lateral sclerosis, comprising administering a morphogen comprising a dimeric protein having an amino acid sequence selected from the group consisting of a sequence:
- (a) having at least 70% homology with the C-terminal seven-cysteine skeleton of human OP-1, residues 330-431 of SEQ ID NO: 2;

- (b) having greater than 60% amino acid sequence identity with said C-terminal seven-cysteine skeleton of human OP-1;
- (c) defined by Generic Sequence 7, SEQ ID NO: 4;
- (d) defined by Generic Sequence 8, SEQ ID NO: 5;
- (e) defined by Generic Sequence 9, SEQ ID NO: 6;
- (f) defined by Generic Sequence 10, SEQ ID NO: 7, and
- (g) defined by OPX, SEQ ID NO: 3,

wherein said morphogen stimulates production of an N-CAM or L1 isoform by an NG108-15 cell *in vitro*.

89. A method of restoring motor function in a mammal afflicted with multiple sclerosis, comprising administering a morphogen comprising a dimeric protein having an amino acid sequence selected from the group consisting of a sequence:

- (a) having at least 70% homology with the C-terminal seven-cysteine skeleton of human OP-1, residues 330-431 of SEQ ID NO: 2;
- (b) having greater than 60% amino acid sequence identity with said C-terminal seven-cysteine skeleton of human OP-1;
- (c) defined by Generic Sequence 7, SEQ ID NO: 4;
- (d) defined by Generic Sequence 8, SEQ ID NO: 5;
- (e) defined by Generic Sequence 9, SEQ ID NO: 6;
- (f) defined by Generic Sequence 10, SEQ ID NO: 7, and
- (g) defined by OPX, SEQ ID NO: 3,

wherein said morphogen stimulates production of an N-CAM or L1 isoform by an NG108-15 cell *in vitro*.

90. A method of restoring motor function in a mammal afflicted with a spinal cord injury, comprising administering a morphogen comprising a dimeric protein having an amino acid sequence selected from the group consisting of a sequence:

- (a) having at least 70% homology with the C-terminal seven-cysteine skeleton of human OP-1, residues 330-431 of SEQ ID NO: 2;

- (b) having greater than 60% amino acid sequence identity with said C-terminal seven-cysteine skeleton of human OP-1;
- (c) defined by Generic Sequence 7, SEQ ID NO: 4;
- (d) defined by Generic Sequence 8, SEQ ID NO: 5;
- (e) defined by Generic Sequence 9, SEQ ID NO: 6;
- (f) defined by Generic Sequence 10, SEQ ID NO: 7, and
- (g) defined by OPX, SEQ ID NO: 3,

wherein said morphogen stimulates production of an N-CAM or L1 isoform by an NG108-15 cell *in vitro*.

91. A method of preserving motor function in a mammal afflicted with or at risk of amyotrophic lateral sclerosis, comprising administering a morphogen comprising a dimeric protein having an amino acid sequence selected from the group consisting of a sequence:

- (a) having at least 70% homology with the C-terminal seven-cysteine skeleton of human OP-1, residues 330-431 of SEQ ID NO: 2;
- (b) having greater than 60% amino acid sequence identity with said C-terminal seven-cysteine skeleton of human OP-1;
- (c) defined by Generic Sequence 7, SEQ ID NO: 4;
- (d) defined by Generic Sequence 8, SEQ ID NO: 5;
- (e) defined by Generic Sequence 9, SEQ ID NO: 6;
- (f) defined by Generic Sequence 10, SEQ ID NO: 7, and
- (g) defined by OPX, SEQ ID NO: 3,

wherein said morphogen stimulates production of an N-CAM or L1 isoform by an NG108-15 cell *in vitro*.

92. A method of preserving motor function in a mammal afflicted with or at risk of multiple sclerosis, comprising administering a morphogen comprising a dimeric protein having an amino acid sequence selected from the group consisting of a sequence:

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- (a) having at least 70% homology with the C-terminal seven-cysteine skeleton of human OP-1, residues 330-431 of SEQ ID NO: 2;
- (b) having greater than 60% amino acid sequence identity with said C-terminal seven-cysteine skeleton of human OP-1;
- (c) defined by Generic Sequence 7, SEQ ID NO: 4;
- (d) defined by Generic Sequence 8, SEQ ID NO: 5;
- (e) defined by Generic Sequence 9, SEQ ID NO: 6;
- (f) defined by Generic Sequence 10, SEQ ID NO: 7, and
- (g) defined by OPX, SEQ ID NO: 3,

wherein said morphogen stimulates production of an N-CAM or L1 isoform by an NG108-15 cell *in vitro*.

93. A method of preserving motor function in a mammal afflicted with or at risk of a spinal cord injury, comprising administering a morphogen comprising a dimeric protein having an amino acid sequence selected from the group consisting of a sequence:

- (a) having at least 70% homology with the C-terminal seven-cysteine skeleton of human OP-1, residues 330-431 of SEQ ID NO: 2;
- (b) having greater than 60% amino acid sequence identity with said C-terminal seven-cysteine skeleton of human OP-1;
- (c) defined by Generic Sequence 7, SEQ ID NO: 4;
- (d) defined by Generic Sequence 8, SEQ ID NO: 5;
- (e) defined by Generic Sequence 9, SEQ ID NO: 6;
- (f) defined by Generic Sequence 10, SEQ ID NO: 7, and
- (g) defined by OPX, SEQ ID NO: 3,

wherein said morphogen stimulates production of an N-CAM or L1 isoform by an NG108-15 cell *in vitro*.

94. A method of treating amyotrophic lateral sclerosis, comprising administering a morphogen selected from the group consisting of human OP-1, mouse OP-1,

human OP-2, mouse OP-2, 60A, GDF-1, BMP2A, BMP2B, DPP, Vgl, Vgr-1, BMP3, BMP5, and BMP6, wherein said morphogen stimulates production of an N-CAM or L1 isoform by an NG108-15 cell *in vitro*.

95. A method of treating multiple sclerosis, comprising administering a morphogen selected from the group consisting of human OP-1, mouse OP-1, human OP-2, mouse OP-2, 60A, GDF-1, BMP2A, BMP2B, DPP, Vgl, Vgr-1, BMP3, BMP5, and BMP6, wherein said morphogen stimulates production of an N-CAM or L1 isoform by an NG108-15 cell *in vitro*.
96. A method of treating a spinal cord injury, comprising administering a morphogen selected from the group consisting of human OP-1, mouse OP-1, human OP-2, mouse OP-2, 60A, GDF-1, BMP2A, BMP2B, DPP, Vgl, Vgr-1, BMP3, BMP5, and BMP6, wherein said morphogen stimulates production of an N-CAM or L1 isoform by an NG108-15 cell *in vitro*.
97. A method of restoring motor function in a mammal afflicted with amyotrophic lateral sclerosis, comprising administering a morphogen selected from the group consisting of human OP-1, mouse OP-1, human OP-2, mouse OP-2, 60A, GDF-1, BMP2A, BMP2B, DPP, Vgl, Vgr-1, BMP3, BMP5, and BMP6, wherein said morphogen stimulates production of an N-CAM or L1 isoform by an NG108-15 cell *in vitro*.
98. A method of restoring motor function in a mammal afflicted with multiple sclerosis, comprising the step of administering a morphogen selected from the group consisting of human OP-1, mouse OP-1, human OP-2, mouse OP-2, 60A, GDF-1, BMP2A, BMP2B, DPP, Vgl, Vgr-1, BMP3, BMP5, and BMP6, wherein said morphogen stimulates production of an N-CAM or L1 isoform by an NG108-15 cell *in vitro*.
99. A method of restoring motor function in a mammal afflicted with a spinal cord injury, comprising administering a morphogen selected from the group consisting of human OP-1, mouse OP-1, human OP-2, mouse OP-2, 60A, GDF-1, BMP2A,

- BMP2B, DPP, Vgl, Vgr-1, BMP3, BMP5, and BMP6, wherein said morphogen stimulates production of an N-CAM or L1 isoform by an NG108-15 cell *in vitro*.
100. A method of preserving motor function in a mammal afflicted with or at risk of amyotrophic lateral sclerosis, comprising administering a morphogen selected from the group consisting of human OP-1, mouse OP-1, human OP-2, mouse OP-2, 60A, GDF-1, BMP2A, BMP2B, DPP, Vgl, Vgr-1, BMP3, BMP5, and BMP6, wherein said morphogen stimulates production of an N-CAM or L1 isoform by an NG108-15 cell *in vitro*.
101. A method of preserving motor function in a mammal afflicted with or at risk of multiple sclerosis, comprising administering a morphogen selected from the group consisting of human OP-1, mouse OP-1, human OP-2, mouse OP-2, 60A, GDF-1, BMP2A, BMP2B, DPP, Vgl, Vgr-1, BMP3, BMP5, and BMP6, wherein said morphogen stimulates production of an N-CAM or L1 isoform by an NG108-15 cell *in vitro*.
102. A method of preserving motor function in a mammal afflicted with or at risk of a spinal cord injury, comprising administering a morphogen selected from the group consisting of human OP-1, mouse OP-1, human OP-2, mouse OP-2, 60A, GDF-1, BMP2A, BMP2B, DPP, Vgl, Vgr-1, BMP3, BMP5, and BMP6, wherein said morphogen stimulates production of an N-CAM or L1 isoform by an NG108-15 cell *in vitro*.
103. The method of claim 82, 83, 84, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101 and 102, wherein said morphogen is complexed with at least one pro-domain polypeptide selected from the group consisting of the pro-domains of OP-1, OP-2, 60A, GDF-1, BMP-2A, BMP-2B, DPP, Vgl, Vgr-1, BMP-3, BMP-5, and BMP-6.
104. The method of claim 103, wherein said morphogen is complexed with a pair of said pro-domain polypeptides.--

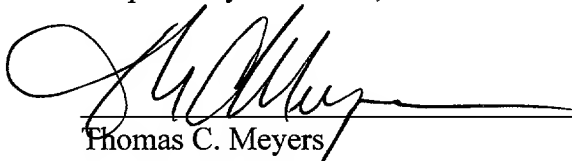
REMARKS

Upon entry of the foregoing amendments, claims 82-104 are pending in the application. Claim 1 is cancelled in the present Preliminary Amendment. Claims 2-81 are cancelled in the Transmittal of Filing Under 37 C.F.R. 1.60(b). New claims 82-104 are fully supported by the Specification as originally filed.

Applicant respectfully submit that the present continuation application, as amended herein, should be in condition for allowance. If the Examiner believes that a telephone conversation with Applicant's attorney would be helpful in expediting prosecution of this application, the Examiner is invited to call the undersigned attorney at (617) 248-7013.

Respectfully submitted,

Dated: September 25, 1997



Thomas C. Meyers

Reg. No. 36,989

Attorney for Applicants

TESTA, HURWITZ & THIBEAULT, LLP
High Street Tower
125 High Street
Boston, MA 02110
Tel: 617-248-7013
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389MM2054/39.413033_1

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EXPRESS MAIL MAILING LABEL

Em354411625US

PATENT

Atty. Docket No.: CRP-070

COMBINED DECLARATION AND POWER OF ATTORNEY
FOR PATENT APPLICATION

(Original, Design, National Stage of PCT, Supplemental,
Divisional, Continuation or CIP)

As a below named inventor, I hereby declare that:

TYPE OF DECLARATION

This declaration is of the following type: (check one
applicable item below)

- ☒ original
☐ design
☐ supplemental
☐ national stage of PCT
☐ divisional
☐ continuation
☐ continuation-in-part (CIP)

INVENTORSHIP IDENTIFICATION

My residence, post office address and citizenship are as
stated next to my name, I believe I am the original, first and
sole inventor (if only one name is listed below) or an original,
first and joint inventor (if plural names are listed below) of
the subject matter which is claimed and for which a patent is
sought on the invention entitled:

TITLE OF INVENTION

MORPHOGEN-INDUCED NERVE REGENERATION AND REPAIR

the specification of which (check one):

- ☒ is attached hereto.
- ☐ was filed on _____ as
Application Serial No. _____ or
- ☐ Express Mail No., as Serial No. not yet known
and was amended on _____
(if applicable).

_____ was described and claimed in PCT International
Application No. _____ filed on _____
and as amended under PCT Article 19 on _____
(if any).

ACKNOWLEDGEMENT OF REVIEW OF PAPERS AND DUTY OF CANDOR

I hereby state that I have reviewed and understand the contents of the specification, including the claims as amended by any amendment referred to herein.

I acknowledge the duty to disclose information which is material to the examination of this application with Title 37, Code of Federal Regulations, §1.56(a).

☐ In compliance with this duty there is attached an information disclosure statement. 37 CFR 1.97.

PRIORITY CLAIM

I hereby claim foreign priority benefits under Title 35, United States, §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed.

Check one:

- ☒ no such applications have been filed.
- ☐ such applications have been filed as follows:

EARLIEST FOREIGN APPLICATION(S), IF ANY FILED WITHIN
12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO
THIS U.S. APPLICATION

Country	Application Number	Date of Filing (mo., day, year)	Priority Claimed Under 37 USC 119	
WO	PCT/US92/01968	March 11, 1992	<input type="checkbox"/> YES	<input checked="" type="checkbox"/> NO <input type="checkbox"/> X
			<input type="checkbox"/> YES	<input type="checkbox"/> NO <input type="checkbox"/>
			<input type="checkbox"/> YES	<input type="checkbox"/> NO <input type="checkbox"/>

ALL FOREIGN APPLICATION(S), IF ANY FILED MORE THAN
12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO
THIS U.S. APPLICATION

CLAIM FOR BENEFIT OF EARLIER U.S./PCT APPLICATION(S)

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application. As to subject matter of this application which is common to my earlier United States application, if any, described below, I do not believe that the same was ever known or used in the United States before my invention thereof or patented or described in any printed publication in any country before my invention thereof or more than one year prior to said earlier application, or in public use or on sale in the United States more than one year prior to said earlier application, that the said common subject matter has not been patented or made the subject of an inventor's certificate

issued before the date of said earlier application in any country foreign to the United States on an application, filed by me or my legal representatives or assigns more than twelve months prior to said application and that no application for patent or inventor's certificate on said subject matter has been filed by me or my representatives or assigns in any country foreign to the United States except those identified herein.

<u>753,059</u> (Application Serial No.)	<u>August 30, 1991</u> (Filing Date)	<u>pending</u> (Status)
<u>667,274</u> (Application Serial No.)	<u>March 11, 1991</u> (Filing Date)	<u>pending</u> (Status) (patented, pending, aband.)
<u>752,764</u> (Application Serial No.)	<u>August 30, 1991</u> (Filing Date)	<u>pending</u> (Status) (patented, pending, aband.)

POWER OF ATTORNEY

As a named inventor, I hereby appoint the following attorneys and/or agents to prosecute this application and transact all business in the United States Patent and Trademark Office connected therewith:

Edmund R. Pitcher,	Reg. No. 27,829
Steven M. Bauer,	Reg. No. 31,481
Paula A. Campbell,	Reg. No. 32,503
Kathleen A. Williams,	Reg. No. 34,380
Robin D. Kelley,	Reg. No. 34,637
Kristofer E. Elbing,	Reg. No. 34,590
Robert J. Tosti,	Reg. No. 35,393
Madeline I. Johnston,	Reg. No. P-36,174

Direct correspondence to:

Patent Administrator
Creative BioMolecules, Inc.

35 South Street
Hopkinton, Massachusetts 01748 U.S.A.

Direct telephone calls to: Robin D. Kelley
(617) 248-7477

(name and telephone number)

Wherefore I petition that letters patent be granted to me for the invention or discovery described and claimed in the attached specification and claims, and hereby subscribe my name to said specification and claims and to the foregoing declaration, power of attorney, and this petition.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

David C. Rueger
Full name of sole or first inventor

David C. Rueger
Inventor's signature

Hopkinton, Massachusetts
Residence

19 Downey Street/Hopkinton, MA 01748
Post Office Address

U.S.A.
Citizenship

7/30/92
Date

Thangavel Kuberasampath
Full name of inventor

Thangavel Kuberasampath
Inventor's signature

Medway, Massachusetts
Residence

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Post Office Address

India
Citizenship

7/30/92
Date

443260-9544630

CHECK PROPER BOX(ES) FOR ANY OF THE FOLLOWING ADDED PAGE(S)
WHICH FORM A PART OF THIS DECLARATION

- ☒ Signature for third and subsequent joint inventors.
Number of pages added 2.
- ☐ Signature by administrator(trix), executor(trix) or
legal representative for deceased or incapacitated
inventor. Number of pages added ____.
- ☐ Signature for inventor who refuses to sign or cannot
be reached by person authorized under 37 CFR 1.47.
Number of pages added ____.
- ☐ Added pages to combined declaration and power of
attorney for divisional, continuation, or continuation
in-part (CIP) application. ____ Number of pages added.
- ☐ Authorization of attorney(s) to accept and follow
instructions from representative.

If no further pages form a part of this Declaration then end this
Declaration with this page and check the following item.

- ☐ This declaration ends this page.

ADDED PAGES FOR ADDITIONAL INVENTORS

Hermann Oppermann
Full name of inventor


Inventor's signature

Medway, Massachusetts
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Post Office Address

U.S.A.
Citizenship

7.30.92
Date

Engin Ozkaynak
Full name of inventor


Inventor's signature

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U.S.A.
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7.30.92
Date

Roy H. L. Pang
Full name of inventor

Inventor's signature

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Citizenship

Date

44 39 60 " 33 60 33 60

ADDED PAGES FOR ADDITIONAL INVENTORS

Hermann Oppermann
Full name of inventor

U.S.A.
Citizenship

Inventor's signature

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Engin Ozkaynak
Full name of inventor

U.S.A.
Citizenship

Inventor's signature

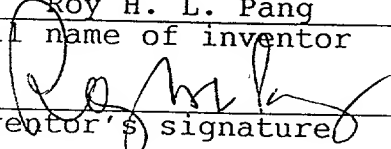
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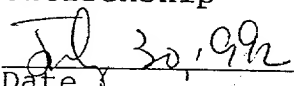
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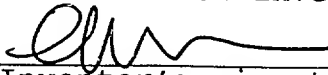
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PATENT

Attorney's Docket No. CRP-070

Applicant or Patentee: David C. Rueger et al.

Serial or Patent No.: 0 / _____

Filed or Issued: July 31, 1992

For: Morphogen-Induced Nerve Regeneration and Repair

**VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY
STATUS (37 CFR 1.9(f) and 1.27(c))—SMALL BUSINESS CONCERN**

I hereby declare that I am

- ☐ the owner of the small business concern identified below:
☒ an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF CONCERN Creative BioMolecules, Inc.

ADDRESS OF CONCERN 35 South Street
Hopkinton, Massachusetts 01748

I hereby declare that the above identified small business concern qualifies as a small business concern as defined in 13 CFR 121.3-18, and reproduced in 37 CFR 1.9(d), for purposes of paying reduced fees under Section 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third-party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed, to and remain with the small business concern identified above with regard to the invention, entitled

Morphogen-Induced Nerve Regeneration and Repair

by inventor(s) David C. Rueger, Thangavel Kuberasampath,
Hermann Oppermann, Engin Ozkaynak, Roy H.L. Pang, Charles M. Cohen

described in

- ☒ the specification filed herewith.
☐ application serial no. 0 / _____, filed _____.
☐ patent no. _____, issued _____.

If the rights held by the above identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 CFR 1.9(d) or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

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I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small business entity is no longer appropriate. (37 CFR 1.28(b)).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING Charles M. Cohen

TITLE OF PERSON OTHER THAN OWNER Chairman and Chief Scientific Officer

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SIGNATURE  Date July 30, 1992

Morphogen-Induced Nerve Regeneration and Repair

5

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of USSN
752,764 and copending USSN 753,059, both filed
10 August 30, 1991 and both continuations-in-part of USSN
667,274, filed March 11, 1991.

BACKGROUND OF THE INVENTION

15 The present invention relates to methods for
enhancing the survival of neuronal cells in vivo and to
methods, compositions and devices for maintaining
neural pathways in vivo. More particularly, the
invention provides methods for enhancing survival of
20 neuronal cells at risk of dying, including methods for
redifferentiating transformed cells of neural origin
and methods for maintaining phenotypic expression of
differentiated neuronal cells. The invention also
provides means for repairing damaged neural pathways,
25 including methods for stimulating axonal growth over
extended distances, and methods for alleviating
immunologically-related nerve tissue damage. In a
particular embodiment of the invention, this invention
provides a method for stimulating cell adhesion
30 molecule expression in cells, and particularly nerve
cell adhesion molecule expression in neurons. Finally,
the invention provides means for evaluating nerve
tissue stasis and identifying neural dysfunction in a
mammal.

35

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The mammalian nervous system comprises a peripheral nervous system (PNS) and a central nervous system (CNS, comprising the brain and spinal cord), and is composed of two principal classes of cells: neurons and glial cells. The glial cells fill the spaces between neurons, nourishing them and modulating their function. Certain glial cells, such as Schwann cells in the PNS and oligodendrocytes in the CNS, also provide a protective myelin sheath that surrounds and protects neuronal axons, which are the processes that extend from the neuron cell body and through which the electric impulses of the neuron are transported. In the peripheral nervous system, the long axons of multiple neurons are bundled together to form a nerve or nerve fiber. These, in turn, may be combined into fascicles, wherein the nerve fibers form bundles embedded, together with the intraneural vascular supply, in a loose collagenous matrix bounded by a protective multilamellar sheath. In the central nervous system, the neuron cell bodies are visually distinguishable from their myelin-ensheathed processes, and are referenced in the art as grey and white matter, respectively.

During development, differentiating neurons from the central and peripheral nervous systems send out axons that must grow and make contact with specific target cells. In some cases, growing axons must cover enormous distances; some grow into the periphery, whereas others stay confined within the central nervous system. In mammals, this stage of neurogenesis is complete during the embryonic phase of life and neuronal cells do not multiply once they have fully differentiated.

Accordingly, the neural pathways of a mammal are particularly at risk if neurons are subjected to mechanical or chemical trauma or to neuropathic degeneration sufficient to put the neurons that define the pathway at risk of dying. A host of neuropathies, some of which affect only a subpopulation or a system of neurons in the peripheral or central nervous systems have been identified to date. The neuropathies, which may affect the neurons themselves or the associated glial cells, may result from cellular metabolic dysfunction, infection, exposure to toxic agents, autoimmunity dysfunction, malnutrition or ischemia. In some cases the cellular dysfunction is thought to induce cell death directly. In other cases, the neuropathy may induce sufficient tissue necrosis to stimulate the body's immune/inflammatory system and the mechanisms of the body's immune response to the initial neural injury then destroys the neurons and the pathway defined by these neurons.

20

Currently no satisfactory method exists to repair the damage caused by these neuropathies, which include multiple sclerosis, amyotrophic lateral sclerosis (ALS), Huntington's chorea, Alzheimer's disease, Parkinson's disease (parkinsonism), and metabolically derived disorders, such as hepatic encephalopathy. Current attempts to counteract the effects of severe traumatic or neural degenerative lesions of the brain and/or spinal cord have to date primarily involved implantation of embryonic neurons in an effort to replace functionally, or otherwise compensate for, lost or deficient neurons. Currently, however, human fetal cell transplantation research is severely restricted. Administration of neurotrophic factors such as nerve growth factor and insulin-like growth factor also have

been suggested to stimulate neuronal growth within the CNS. (See, for example, Lundborg, (1987) Acta Orthop. Scand. 58:145-169 and US Pat. No. 5,093,317.)

Administration of neurotrophic factors to the CNS
5 requires bypassing the blood-brain barrier. The barrier may be overcome by direct infusion, or by modifying the molecule to enhance its transport across the barrier, as by chemical modification or conjugation, or by molecule truncation. Schwann cells
10 also have been grafted to a site of a CNS lesion in an attempt to stimulate and maintain growth of damaged neuronal processes (Paino et al. (1991) Exp. Neurology 114(2):254-257).

15 Where the damaged neural pathway results from CNS axonal damage, autologous peripheral nerve grafts have been used to bridge lesions in the central nervous system and to allow axons to make it back to their normal target area. In contrast to CNS neurons, neurons
20 of the peripheral nervous system can extend new peripheral processes in response to axonal damage. This regenerative property of peripheral nervous system axons is thought to be sufficient to allow grafting of these segments to CNS axons. Successful grafting
25 appears to be limited, however, by a number of factors, including the length of the CNS axonal lesion to be bypassed, and the distance of the graft sites from the CNS neuronal cell bodies, with successful grafts occurring near the cell body.

30 Within the peripheral nervous system, this cellular regenerative property of neurons has limited ability to repair function to a damaged neural pathway.

Specifically, the new axons extend randomly, and are
35 often misdirected, making contact with inappropriate

targets that can cause abnormal function. For example, if a motor nerve is damaged, regrowing axons may contact the wrong muscles, resulting in paralysis. In addition, where severed nerve processes result in a gap of longer than a few millimeters, e.g., greater than 10 millimeters (mm), appropriate nerve regeneration does not occur, either because the processes fail to grow the necessary distance, or because of misdirected axonal growth. Efforts to repair peripheral nerve damage by surgical means has met with mixed results, particularly where damage extends over a significant distance. In some cases, the suturing steps used to obtain proper alignment of severed nerve ends stimulates the formulation of scar tissue which is thought to inhibit axon regeneration. Even where scar tissue formation has been reduced, as with the use of nerve guidance channels or other tubular prostheses, successful regeneration generally still is limited to nerve damage of less than 10 millimeters in distance. In addition, the reparative ability of peripheral neurons is significantly inhibited where an injury or neuropathy affects the cell body itself or results in extensive degeneration of a distal axon.

Mammalian neural pathways also are at risk due to damage caused by neoplastic lesions. Neoplasias of both the neurons and glial cells have been identified. Transformed cells of neural origin generally lose their ability to behave as normal differentiated cells and can destroy neural pathways by loss of function. In addition, the proliferating tumors may induce lesions by distorting normal nerve tissue structure, inhibiting pathways by compressing nerves, inhibiting cerebrospinal

fluid or blood supply flow, and/or by stimulating the body's immune response. Metastatic tumors, which are a significant cause of neoplastic lesions in the brain and spinal cord, also similarly may damage neural pathways and induce neuronal cell death.

One type of morphoregulatory molecule associated with neuronal cell growth, differentiation and development is the cell adhesion molecule ("CAM"), most notably the nerve cell adhesion molecule (N-CAM). CAMs belong to the immunoglobulin super-family and mediate cell-cell interactions in developing and adult tissues through homophilic binding, i.e., CAM-CAM binding on apposing cells. A number of different CAMs currently have been identified. Of these, the most thoroughly studied to date are N-CAM and L-CAM (liver cell adhesion molecules), both of which have been identified on all cells at early stages of development, as well as in different adult tissues. In neural tissue development, N-CAM expression is believed to be important in tissue organization, neuronal migration, nerve-muscle tissue adhesion, retinal formation, synaptogenesis, and neural degeneration. Reduced N-CAM expression also is thought to be associated with nerve dysfunction. For example, expression of at least one form of N-CAM, N-CAM-180, is reduced in a mouse dysmyelinating mutant (Bhat (1988) Brain Res. 452:373-377). Reduced levels of N-CAM also have been associated with normal pressure hydrocephalus (Werdelin (1989) Acta Neurol. Scand. 79:177-181), and with type II schizophrenia (Lyons et al., (1988) Biol. Psychiatry 23:769-775.) In addition, antibodies to N-CAM have been shown to disrupt functional recovery in injured nerves (Remsen (1990) Exp. Neurobiol. 110:268-273).

It is an object of this invention to provide methods for enhancing survival of neurons at risk of dying in a mammal. Another object is to provide methods for maintaining neural pathways in vivo at risk of injury, or following damage to nerve tissue due to mechanical or chemical trauma, a neuropathy, or a neoplastic lesion. Another object is to provide compositions and devices for repairing gaps in a neural pathway of the peripheral nervous system. Yet another object is to provide a means for redifferentiating transformed cells defining neural pathways, particularly transformed cells of neural origin. Another object is to provide a means for stimulating CAM expression, particularly N-CAM expression in a cell. Yet another object is to provide methods for monitoring the status of nerve tissue by monitoring fluctuations in protein levels present in nerve tissue, serum and/or cerebrospinal fluid. These and other objects and features of the invention will be apparent from the description, drawings, and claims which follow.

Summary of the Invention

The present invention provides methods and
5 compositions for maintaining neural pathways in a
mammal in vivo, including methods for enhancing the
survival of neural cells.

In one aspect, the invention features compositions
10 and therapeutic treatment methods that comprise the
step of administering to a mammal a therapeutically
effective amount of a morphogenic protein
("morphogen"), as defined herein, upon injury to a
neural pathway, or in anticipation of such injury, for
15 a time and at a concentration sufficient to maintain
the neural pathway, including repairing damaged
pathways, or inhibiting additional damage thereto.

In another aspect, the invention features
20 compositions and therapeutic treatment methods for
maintaining neural pathways in a mammal in vivo which
include administering to the mammal, upon injury to a
neural pathway or in anticipation of such injury, a
compound that stimulates in vivo a therapeutically
25 effective concentration of an endogenous morphogen
within the body of the mammal sufficient to maintain
the neural pathway, including repairing damaged
pathways or inhibiting additional damage thereto.
These compounds are referred to herein as morphogen-
30 stimulating agents, and are understood to include
substances which, when administered to a mammal, act on

tissue(s) or organ(s) that normally are responsible for, or capable of, producing a morphogen and/or secreting a morphogen, and which cause the endogenous level of the morphogen to be altered. The agent may
5 act, for example, by stimulating expression and/or secretion of an endogenous morphogen.

In particular, the invention provides methods for enhancing the survival of neurons at risk of dying,
10 including protecting neurons from the tissue destructive effects associated with the body's immune/inflammatory response to a nerve injury. The invention also provides methods for stimulating neurons to maintain their differentiated phenotype, including
15 inducing the redifferentiation of transformed cells of neuronal origin to a morphology characteristic of untransformed neurons. In one embodiment, the invention provides means for stimulating production of cell adhesion molecules in cells, particularly nerve
20 cell adhesion molecules (N-CAM) in neurons. The invention also provides methods, compositions and devices for stimulating cellular repair of damaged neurons and neural pathways, including regenerating damaged axons of the peripheral and central nervous
25 systems. In addition, the invention also provides means for evaluating the status of nerve tissue, and for detecting and monitoring neuropathies in a mammal by monitoring fluctuations in the morphogen levels or endogenous morphogen antibody levels present in a
30 mammal's serum or cerebrospinal fluid.

As used herein, a "neural pathway" describes a nerve circuit for the passage of electric signals from a source to a target cell site. The pathway includes
35 the neurons through which the electric impulse is

transported, including groups of interconnecting neurons, the nerve fibers formed by bundled neuronal axons, and the glial cells surrounding and associated with the neurons.

5

In one aspect of the invention, the morphogens described herein are useful in repairing damaged neural pathways of the peripheral nervous system. In particular, the morphogens are useful for repairing
10 damaged pathways, including transected or otherwise damaged nerve fibers (nerves) requiring regeneration of neuronal processes, particularly axons, over extended distances to bridge a gap in the nerve itself, or between the nerve and a post-synaptic cell.
15 Specifically, the morphogens described herein are capable of stimulating complete axonal nerve regeneration, including vascularization and reformation of the protective myelin sheath. The morphogen preferably is provided to the site of injury dispersed
20 in a biocompatible, bioresorbable carrier material capable of maintaining the morphogen at the site and, where necessary, means for directing axonal growth from the proximal to the distal ends of a severed neuron or nerve. For example, means for directing axonal growth
25 may be required where nerve regeneration is to be induced over an extended distance, such as greater than 10 mm. Many carriers capable of providing these functions are envisioned. For example, useful carriers include substantially insoluble materials or viscous
30 solutions prepared as disclosed herein comprising laminin, hyaluronic acid or collagen, or other suitable synthetic, biocompatible polymeric materials such as

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polylactic, polyglycolic or polybutyric acids and/or copolymers thereof. The currently preferred carrier comprises an extracellular matrix composition, such as one described herein derived, for example, from mouse
5 sarcoma cells. Also envisioned as especially useful are brain tissue-derived extracellular matrices.

In a particularly preferred embodiment, the morphogen is provided to the site as part of a device
10 wherein the morphogen is disposed in a nerve guidance channel which spans the distance of the damaged pathway. The channel acts both as a protective covering and a physical means for guiding growth of a neuronal process such as an axon. Useful channels
15 comprise a biocompatible membrane or casing, which may be tubular in structure, having a dimension sufficient to span the gap or break in the nerve to be repaired, and having openings adapted to receive severed nerve ends. The casing or membrane may be made of any
20 biocompatible, nonirritating material, such as silicone or a biocompatible polymer such as polyethylene or polyethylene vinyl acetate. The casing also may be composed of biocompatible, bioresorbable polymers, including, for example, collagen, hyaluronic acid,
25 polylactic, polybutyric and polyglycolic acids. In a currently preferred embodiment, the outer surface of the channel is substantially impermeable.

The morphogen may be disposed in the channel in
30 association with a biocompatible carrier material, or it may be adsorbed to or otherwise associated with the inner surface of the casing, such as is described in U.S. Pat. No. 5,011,486, provided that the morphogen is accessible to the severed nerve ends. Additionally,
35 although the nerve guidance channels described herein

generally are tubular in shape, it should be evident to those skilled in the art that various alternative shapes may be employed. The lumen of the guidance channels may, for example, be oval or even square in cross section. Moreover the guidance channels may be constructed of two or more parts which may be clamped together to secure the nerve stumps. Nerve endings may be secured to the nerve guidance channels by means of sutures, biocompatible adhesives such as fibrin glue, or other means known in the medical art.

The morphogens described herein also are envisioned to be useful in autologous peripheral nerve segment implants to bypass damaged neural pathways in the central nervous system, such as in the repair of damaged or detached retinas, or other damage to the optic nerve. Here the morphogen is provided to the site of attachment to stimulate axonal growth at the graft site, particularly where the damaged axonal segment to be bypassed occurs far from the neuronal cell body.

The morphogens described herein also are useful for enhancing survival of neuronal cells at risk of dying, thereby preventing, limiting or otherwise inhibiting damage to neural pathways. Non-mitotic neurons are at risk of dying as a result of a neuropathy or other cellular dysfunction of a neuron or glial cell inducing cell death, or following a chemical or mechanical lesion to the cell or its surrounding tissue. The chemical lesions may result from known toxic agents, including lead, ethanol, ammonia, formaldehyde and many other organic solvents, as well as the toxins in cigarette smoke and opiates. Excitatory amino acids, such as glutamate also may play a role in the

pathogenesis of neuronal cell death (see Freese et al. (1990) Brain Res. 521:254-264). Neuronal cell death also is thought to be a significant contributing factor in a number of neurodegenerative diseases, including

5 Alzheimer's disease, Huntington's chorea, and Parkinson's disease, amyotrophic lateral sclerosis and multiple sclerosis. The etiology of these neuropathies may be metabolic, as results in hepatic encephalopathy, infectious, toxic, autoimmune, nutritional or ischemic.

10 In addition, ethanol and a number of other toxins also have been identified as significant contributing factors in neurodegenerative diseases. The morphogens described herein may be provided to cells at risk of dying to enhance their survival and thereby protect the

15 integrity of the neural pathway. The morphogens may be provided directly to the site, or they may be provided systemically. Alternatively, as described above, an agent capable of stimulating endogenous morphogen expression and/or secretion, preferably in cells

20 associated with the nerve tissue of interest, may be administered to the mammal.

In another aspect of the invention, the method disclosed is useful for redifferentiating transformed

25 cells, particularly transformed cells of neuronal or glial origin, such that the morphogen-treated cells are induced to display a morphology characteristic of untransformed cells. Where the transformed cells are cells of neuronal origin, morphogen treatment

30 preferably induces cell rounding and cell aggregation (clumping), cell-cell adhesion, neurite outgrowth formation and elongation, and N-CAM production. The methods described herein are anticipated to substantially inhibit or reduce neural cell tumor

35 formation and/or proliferation in nerve tissue. It is

anticipated that the methods of this invention will be
useful in substantially reducing the effects of various
carcinomas of nerve tissue origin such as
retinoblastomas, neuroblastomas, and gliomas or
5 glioblastomas. In addition, the method also is
anticipated to aid in inhibiting neoplastic lesions
caused by metastatic tissue. Metastatic tumors are one
of the most common neoplasms of the CNS, as they can
reach the intracranial compartment through the
10 bloodstream. Metastatic tumors may damage neural
pathways for example, by distorting normal nerve tissue
structure, compressing nerves, blocking flow of
cerebrospinal fluid or the blood supply nourishing
brain tissue, and/or by stimulating the body's immune
15 response.

In another aspect of the invention, the morphogens
described herein are useful for providing
neuroprotective effects to alleviate neural pathway
20 damage associated with the body's immune/inflammatory
response to an initial injury to nerve tissue. Such a
response may follow trauma to nerve tissue, caused, for
example, by an autoimmune dysfunction, neoplastic
lesion, infection, chemical or mechanical trauma,
25 disease, by interruption of blood flow to the neurons
or glial cells, for example following ischemia or
hypoxia, or by other trauma to the nerve or surrounding
material. For example, the primary damage resulting
from hypoxia or ischemia-reperfusion following
30 occlusion of a neural blood supply, as in an embolic
stroke, is believed to be immunologically associated.
In addition, at least part of the damage associated
with a number of primary brain tumors also appears to
be immunologically related. Application of the
35 morphogen directly to the cells to be treated, or

providing the morphogen to the mammal systemically, for example, intravenously or indirectly by oral administration, may be used to alleviate and/or inhibit the immunologically related response to a neural injury. Alternatively, administration of an agent capable of stimulating morphogen expression and/or secretion in vivo, preferably at the site of injury, also may be used. Where the injury is to be induced, as during surgery or other aggressive clinical treatment, the morphogen or agent may be provided prior to induction of the injury to provide a neuroprotective effect to the nerve tissue at risk.

In still another aspect, the invention described herein provides methods for supporting the growth and maintenance of differentiated neurons, including inducing neurons to continue expressing their phenotype. It is anticipated that this activity will be particularly useful in the treatment of nerve tissue disorders where loss of function is caused by reduced or lost cellular metabolic function and cells become senescent or quiescent, such as is thought to occur in aging cells and to be manifested in Alzheimer's disease. Application of the morphogen directly to cells to be treated, or providing it systemically by parenteral or oral administration stimulates these cells to continue expressing their phenotype, significantly inhibiting and/or reversing the effects of the cellular metabolic dysfunction, thereby maintaining the neural pathway at risk. Alternatively, administration of an agent capable of stimulating endogenous morphogen expression and/or secretion in vivo may be used.

In still another aspect, the invention provides methods for stimulating CAM expression levels in a cell, particularly N-CAM expression in neurons. CAMs are molecules defined as carrying out cell-cell
5 interactions necessary for tissue formation. CAMs are believed to play a fundamental regulatory role in tissue development, including tissue boundary formation, embryonic induction and migration, and tissue stabilization and regeneration. Altered CAM
10 levels have been implicated in a number of tissue disorders, including congenital defects, neoplasias, and degenerative diseases.

In particular, N-CAM expression is associated with
15 normal neuronal cell development and differentiation, including retinal formation, synaptogenesis, and nerve-muscle tissue adhesion. Inhibition of one or more of the N-CAM isoforms is known to prevent proper tissue development. Altered N-CAM expression levels also are
20 associated with neoplasias, including neuroblastomas (see *infra*), as well as with a number of neuropathies, including normal pressure hydrocephalous and type II schizophrenia. Application of the morphogen directly to the cells to be treated, or providing the morphogen
25 to the mammal systemically, for example, parenterally, or indirectly by oral administration, may be used to induce cellular expression of one or more CAMs, particularly N-CAMs. Alternatively, administration of an agent capable of stimulating morphogen expression
30 and/or secretion in vivo, preferably at the site of injury, also may be used to induce CAM production.

CAMs also have been postulated as part of a morphoregulatory pathway whose activity is induced by a
35 to date unidentified molecule (See, for example,

Edelman, G.M. (1986) Ann. Rev. Cell Biol. 2:81-116). Without being limited to any given theory, the morphogens described herein may act as the inducer of this pathway.

5

Finally, modulations of endogenous morphogen levels may be monitored as part of a method of detecting nerve tissue dysfunction. Specifically, modulations in endogenous morphogen levels are anticipated to reflect changes in nerve tissue status. Morphogen expression may be monitored directly in biopsied cell samples, in cerebrospinal fluid, or serum. Alternatively, morphogen levels may be assessed by detecting changes in the levels of endogenous antibodies to the morphogen. For example, one may obtain serum samples from a mammal, and then detect the concentration of morphogen or antibody present in the fluid by standard protein detection means known to those skilled in the art. As an example, binding protein capable of interacting specifically with the morphogen of interest such as an anti-morphogen antibody may be used to detect a morphogen in a standard immunoassay. The morphogen levels detected then may be compared to a previously determined standard or reference level, with changes in the detected levels being indicative of the status of the tissue.

In one preferred embodiment of the invention, the morphogen or morphogen-stimulating agent is administered systemically to the individual, e.g., orally or parenterally. In another embodiment of the invention, the morphogen may be provided directly to the nerve tissue, e.g., by injection to the cerebral spinal fluid or to a nerve tissue locus.

35

In any treatment method of the invention, "administration of morphogen" refers to the administration of the morphogen, either alone or in combination with other molecules. For example, the
5 mature form of the morphogen may be provided in association with its precursor "pro" domain, which is known to enhance the solubility of the protein. Other useful molecules known to enhance protein solubility include casein and other milk components, as well as
10 various serum proteins. Additional useful molecules which may be associated with the morphogen or morphogen-stimulating agent include tissue targeting molecules capable of directing the morphogen or morphogen-stimulating agent to nerve tissue. Tissue
15 targeting molecules envisioned to be useful in the treatment protocols of this invention include antibodies, antibody fragments or other binding proteins which interact specifically with surface molecules on nerve tissue cells.

20

Still another useful tissue targeting molecule is part or all of the morphogen precursor "pro" domain, particularly that of OP-1 or GDF-1. These proteins are found naturally associated with nerve tissue but also
25 may be synthesized in other tissues and targeted to nerve tissue after secretion from the synthesizing tissue. For example, while the protein has been shown to be active in bone tissue, the primary source of OP-1 synthesis appears to be the tissue of the urogenic
30 system (e.g., renal and bladder tissue), with secondary expression levels occurring in the brain, heart and lungs (see below.) Moreover, the protein has been

identified in serum, saliva and various milk forms. In addition, the secreted form of the protein comprises the mature dimer in association with the pro domain of the intact morphogen sequence. Accordingly, the associated morphogen pro domains may act to target specific morphogens to different tissues in vivo.

Associated tissue targeting or solubility-enhancing molecules also may be covalently linked to the morphogen using standard chemical means, including acid-labile linkages, which likely will be preferentially cleaved in the acidic environment of bone remodeling sites.

Finally, the morphogens or morphogen-stimulating agents provided herein also may be administered in combination with other molecules known to be beneficial in maintaining neural pathways, including, for example, nerve growth factors and anti-inflammatory agents.

Where the morphogen is intended for use as a therapeutic for disorders of the CNS, an additional problem must be addressed: overcoming the so-called "blood-brain barrier", the brain capillary wall structure that effectively screens out all but selected categories of molecules present in the blood, preventing their passage into the brain. The blood-brain barrier may be bypassed effectively by direct infusion of the morphogen or morphogen-stimulating agent into the brain. Alternatively, the morphogen or morphogen-stimulating agent may be modified to enhance its transport across the blood-brain barrier. For example, truncated forms of the morphogen or a morphogen-stimulating agent may be most successful. Alternatively, the morphogen or

morphogen-stimulating agent may be modified to render it more lipophilic, or it may be conjugated to another molecule which is naturally transported across the barrier, using standard means known to those skilled in the art, as, for example, described in Pardridge, Endocrine Reviews 7:314-330 (1986) and U.S. Pat. No. 4,801,575.

Accordingly, as used herein, a functional "analog" of a morphogen refers to a protein having morphogenic biological activity but possessing additional structural differences compared to a morphogen as defined herein, e.g., having additional chemical moieties not normally a part of a morphogen. Such moieties (introduced, for example, by acylation, alkylation, cationization, or glycosylation reactions, or other means for conjugating the moiety to the morphogen) may improve the molecule's solubility, absorption, biological half-life, or transport, e.g., across the blood-brain barrier.

Among the morphogens useful in this invention are proteins originally identified as osteogenic proteins, such as the OP-1, OP-2 and CBMP2 proteins, as well as amino acid sequence-related proteins such as DPP (from *Drosophila*), Vgl (from *Xenopus*), Vgr-1 (from mouse, see U.S. 5,011,691 to Oppermann et al.), GDF-1 (from mouse, see Lee (1991) PNAS 88:4250-4254), all of which are presented in Table II and Seq. ID Nos.5-14), and the recently identified 60A protein (from *Drosophila*, Seq. ID No. 24, see Wharton et al. (1991) PNAS 88:9214-9218.) The members of this family, which include members of the TGF- β super-family of proteins, share substantial amino acid sequence homology in their C-terminal regions. The proteins are translated as a

precursor, having an N-terminal signal peptide sequence, typically less than about 30 residues, followed by a "pro" domain that is cleaved to yield the mature sequence. The signal peptide is cleaved rapidly upon translation, at a cleavage site that can be predicted in a given sequence using the method of Von Heijne ((1986) Nucleic Acids Research 14:4683-4691.) Table I, below, describes the various morphogens identified to date, including their nomenclature as used herein, their Seq. ID references, and publication sources for the amino acid sequences for the full length proteins not included in the Seq. Listing. The disclosure of these publications is incorporated herein by reference.

TABLE I

15	"OP-1"	Refers generically to the group of morphogenically active proteins expressed from part or all of a DNA sequence
20		encoding OP-1 protein, including allelic and species variants thereof, e.g., human OP-1 ("hOP-1", Seq. ID No. 5, mature protein amino acid sequence), or mouse OP-1 ("mOP-1", Seq. ID No. 6, mature protein amino acid sequence.)
25		The conserved seven cysteine skeleton is defined by residues 38 to 139 of Seq. ID Nos. 5 and 6. The cDNA sequences and the amino acids encoding the full length
30		proteins are provided in Seq. ID Nos. 16 and 17 (hOP1) and Seq. ID Nos. 18 and 19 (mOP1.) The mature proteins are defined

by residues 293-431 (hOP1) and 292-430 (mOP1). The "pro" regions of the proteins, cleaved to yield the mature, morphogenically active proteins are defined essentially by residues 30-292 (hOP1) and residues 30-291 (mOP1).

"OP-2" refers generically to the group of active proteins expressed from part or all of a DNA sequence encoding OP-2 protein, including allelic and species variants thereof, e.g., human OP-2 ("hOP-2", Seq. ID No. 7, mature protein amino acid sequence) or mouse OP-2 ("mOP-2", Seq. ID No. 8, mature protein amino acid sequence). The conserved seven cysteine skeleton is defined by residues 38 to 139 of Seq. ID Nos. 7 and 8. The cDNA sequences and the amino acids encoding the full length proteins are provided in Seq. ID Nos. 20 and 21 (hOP2) and Seq. ID Nos. 22 and 23 (mOP2.) The mature proteins are defined essentially by residues 264-402 (hOP2) and 261-399 (mOP2). The "pro" regions of the proteins, cleaved to yield the mature, morphogenically active proteins likely are defined essentially by residues 18-263 (hOP2) and residues 18-260 (mOP2). (Another cleavage site also occurs 21 residues upstream for both OP-2 proteins.)

"CBMP2" refers generically to the morphogenically active proteins expressed from a DNA sequence encoding the CBMP2 proteins, including allelic and species variants thereof, e.g., human CBMP2A ("CBMP2A(fx)", Seq ID No. 9) or human CBMP2B DNA ("CBMP2B(fx)", Seq. ID No. 10). The amino acid sequence for the full length proteins, referred to in the literature as BMP2A and BMP2B, or BMP2 and BMP4, appear in Wozney, et al. (1988) Science 242:1528-1534. The pro domain for BMP2 (BMP2A) likely includes residues 25-248 or 25-282; the mature protein, residues 249-396 or 283-396. The pro domain for BMP4 (BMP2B) likely includes residues 25-256 or 25-292; the mature protein, residues 257-408 or 293-408.

20 "DPP(fx)" refers to protein sequences encoded by the Drosophila DPP gene and defining the conserved seven cysteine skeleton (Seq. ID No. 11). The amino acid sequence for the full length protein appears in Padgett, et al (1987) Nature 325: 81-84. The pro domain likely extends from the signal peptide cleavage site to residue 456; the mature protein likely is defined by residues 457-588.

30 "Vgl(fx)" refers to protein sequences encoded by the Xenopus Vgl gene and defining the conserved seven cysteine skeleton (Seq. ID No. 12). The amino acid sequence for the full length protein appears in

Weeks (1987) Cell 51: 861-867. The prodomain likely extends from the signal peptide cleavage site to residue 246; the mature protein likely is defined by residues 247-360.

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"Vgr-1(fx)" refers to protein sequences encoded by the murine Vgr-1 gene and defining the conserved seven cysteine skeleton (Seq. ID No. 13). The amino acid sequence for the full length protein appears in Lyons, et al, (1989) PNAS 86: 4554-4558. The prodomain likely extends from the signal peptide cleavage site to residue 299; the mature protein likely is defined by residues 300-438.

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"GDF-1(fx)" refers to protein sequences encoded by the human GDF-1 gene and defining the conserved seven cysteine skeleton (Seq. ID No. 14). The cDNA and encoded amino sequence for the full length protein is provided in Seq. ID. No. 32. The prodomain likely extends from the signal peptide clavage site to residue 214; the mature protein likely is defined by residues 215-372.

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"60A" refers generically to the morphogenically active proteins expressed from part or all of a DNA sequence (from the Drosophila 60A gene) encoding the 60A proteins (see Seq. ID No. 24 wherein the cDNA and encoded amino acid sequence for the full length protein is provided). "60A(fx)" refers to

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the protein sequences defining the conserved seven cysteine skeleton (residues 354 to 455 of Seq. ID No. 24.) The prodomain likely extends from the signal peptide cleavage site to residue 324; the mature protein likely is defined by residues 325-455.

- 5
- 10 "BMP3(fx)" refers to protein sequences encoded by the human BMP3 gene and defining the conserved seven cysteine skeleton (Seq. ID No. 26). The amino acid sequence for the full length protein appears in Wozney et al. (1988) Science 242: 1528-1534. The pro domain likely extends from the signal peptide cleavage site to residue 290; the mature protein likely is defined by residues 291-472.
- 15
- 20 "BMP5(fx)" refers to protein sequences encoded by the human BMP5 gene and defining the conserved seven cysteine skeleton (Seq. ID No. 27). The amino acid sequence for the full length protein appears in Celeste, et al. (1991) PNAS 87: 9843-9847. The pro domain likely extends from the signal peptide cleavage site to residue 316; the mature protein likely is defined by residues 317-454.
- 25
- 30 "BMP6(fx)" refers to protein sequences encoded by the human BMP6 gene and defining the conserved seven cysteine skeleton (Seq. ID No. 28). The amino acid sequence for the full
- 35 length protein appear sin Celeste, et al.

(1990) PNAS 87: 9843-5847. The pro domain likely includes extends from the signal peptide cleavage site to residue 374; the mature sequence likely includes residues 375-513.

The OP-2 proteins have an additional cysteine residue in this region (e.g., see residue 41 of Seq. ID Nos. 7 and 8), in addition to the conserved cysteine skeleton in common with the other proteins in this family. The GDF-1 protein has a four amino acid insert within the conserved skeleton (residues 44-47 of Seq. ID No. 14) but this insert likely does not interfere with the relationship of the cysteines in the folded structure. In addition, the CBMP2 proteins are missing one amino acid residue within the cysteine skeleton.

The morphogens are inactive when reduced, but are active as oxidized homodimers and when oxidized in combination with other morphogens of this invention. Thus, as defined herein, a morphogen is a dimeric protein comprising a pair of polypeptide chains, wherein each polypeptide chain comprises at least the C-terminal six cysteine skeleton defined by residues 43-139 of Seq. ID No. 5, including functionally equivalent arrangements of these cysteines (e.g., amino acid insertions or deletions which alter the linear arrangement of the cysteines in the sequence but not their relationship in the folded structure), such that, when the polypeptide chains are folded, the dimeric protein species comprising the pair of polypeptide chains has the appropriate three-dimensional structure, including the appropriate intra- or inter-chain disulfide bonds such that the protein is capable of

acting as a morphogen as defined herein. Specifically,
the morphogens generally are capable of all of the
following biological functions in a morphogenically
permissive environment: stimulating proliferation of
5 progenitor cells; stimulating the differentiation of
progenitor cells; stimulating the proliferation of
differentiated cells; and supporting the growth and
maintenance of differentiated cells, including the
"redifferentiation" of transformed cells. In addition,
10 it is also anticipated that these morphogens are
capable of inducing redifferentiation of committed
cells under appropriate environmental conditions.

In one preferred aspect, the morphogens of
15 this invention comprise one of two species of generic
amino acid sequences: Generic Sequence 1 (Seq. ID
No. 1) or Generic Sequence 2 (Seq. ID No. 2); where
each Xaa indicates one of the 20 naturally-occurring
L-isomer, α -amino acids or a derivative thereof.
20 Generic Sequence 1 comprises the conserved six cysteine
skeleton and Generic Sequence 2 comprises the conserved
six cysteine skeleton plus the additional cysteine
identified in OP-2 (see residue 36, Seq. ID No. 2). In
another preferred aspect, these sequences further
25 comprise the following additional sequence at their N-
terminus:

Cys Xaa Xaa Xaa Xaa (Seq. ID No. 15)
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Preferred amino acid sequences within the
foregoing generic sequences include: Generic Sequence
3 (Seq. ID No. 3), Generic Sequence 4 (Seq. ID No. 4),
Generic Sequence 5 (Seq. ID No. 30) and Generic
35 Sequence 6 (Seq. ID No. 31), listed below. These

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Leu Tyr Val Xaa Phe

Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa

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Xaa Ala Pro Xaa Gly Xaa Xaa Ala

15 20

Xaa Tyr Cys Xaa Gly Xaa Cys Xaa
 25 30
 Xaa Pro Xaa Xaa Xaa Xaa Xaa
 35
 5 Xaa Xaa Xaa Asn His Ala Xaa Xaa
 40 45
 Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa
 50
 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
 10 55 60
 Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa
 65
 Xaa Xaa Xaa Leu Xaa Xaa Xaa
 70 75
 15 Xaa Xaa Xaa Xaa Val Xaa Leu Xaa
 80
 Xaa Xaa Xaa Xaa Met Xaa Val Xaa
 85 90
 Xaa Cys Gly Cys Xaa
 20 95

wherein each Xaa is independently selected from a group
 of one or more specified amino acids defined as
 follows: "Res." means "residue" and Xaa at res.4 =
 (Ser, Asp or Glu); Xaa at res.6 = (Arg, Gln, Ser or
 25 Lys); Xaa at res.7 = (Asp or Glu); Xaa at res.8 = (Leu
 or Val); Xaa at res.11 = (Gln, Leu, Asp, His or Asn);
 Xaa at res.12 = (Asp, Arg or Asn); Xaa at res.14 = (Ile
 or Val); Xaa at res.15 = (Ile or Val); Xaa at res.18 =

(Glu, Gln, Leu, Lys, Pro or Arg); Xaa at res.20 = (Tyr or Phe); Xaa at res.21 = (Ala, Ser, Asp, Met, His, Leu or Gln); Xaa at res.23 = (Tyr, Asn or Phe); Xaa at res.26 = (Glu, His, Tyr, Asp or Gln); Xaa at res.28 =
5 (Glu, Lys, Asp or Gln); Xaa at res.30 = (Ala, Ser, Pro or Gln); Xaa at res.31 = (Phe, Leu or Tyr); Xaa at res.33 = (Leu or Val); Xaa at res.34 = (Asn, Asp, Ala or Thr); Xaa at res.35 = (Ser, Asp, Glu, Leu or Ala); Xaa at res.36 = (Tyr, Cys, His, Ser or Ile); Xaa at
10 res.37 = (Met, Phe, Gly or Leu); Xaa at res.38 = (Asn or Ser); Xaa at res.39 = (Ala, Ser or Gly); Xaa at res.40 = (Thr, Leu or Ser); Xaa at res.44 = (Ile or Val); Xaa at res.45 = (Val or Leu); Xaa at res.46 = (Gln or Arg); Xaa at res.47 = (Thr, Ala or Ser); Xaa at
15 res.49 = (Val or Met); Xaa at res.50 = (His or Asn); Xaa at res.51 = (Phe, Leu, Asn, Ser, Ala or Val); Xaa at res.52 = (Ile, Met, Asn, Ala or Val); Xaa at res.53 = (Asn, Lys, Ala or Glu); Xaa at res.54 = (Pro or Ser); Xaa at res.55 = (Glu, Asp, Asn, or Gly); Xaa at res.56
20 = (Thr, Ala, Val, Lys, Asp, Tyr, Ser or Ala); Xaa at res.57 = (Val, Ala or Ile); Xaa at res.58 = (Pro or Asp); Xaa at res.59 = (Lys or Leu); Xaa at res.60 = (Pro or Ala); Xaa at res.63 = (Ala or Val); Xaa at res.65 = (Thr or Ala); Xaa at res.66 = (Gln, Lys, Arg or Glu); Xaa at res.67 = (Leu, Met or Val); Xaa at
25 res.68 = (Asn, Ser or Asp); Xaa at res.69 = (Ala, Pro or Ser); Xaa at res.70 = (Ile, Thr or Val); Xaa at res.71 = (Ser or Ala); Xaa at res.72 = (Val or Met); Xaa at res.74 = (Tyr or Phe); Xaa at res.75 = (Phe, Tyr or Leu); Xaa at res.76 = (Asp or Asn); Xaa at res.77 =
30 (Asp, Glu, Asn or Ser); Xaa at res.78 = (Ser, Gln, Asn or Tyr); Xaa at res.79 = (Ser, Asn, Asp or Glu); Xaa at res.80 = (Asn, Thr or Lys); Xaa at res.82 = (Ile or

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Generic Sequence 4

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wherein each Xaa is independently selected from a group of one or more specified amino acids as defined by the following: "Res." means "residue" and Xaa at res.2 = (Lys or Arg); Xaa at res.3 = (Lys or Arg); Xaa at res.4 = (His or Arg); Xaa at res.5 = (Glu, Ser, His, Gly, Arg or Pro); Xaa at res.9 = (Ser, Asp or Glu); Xaa at res.11 = (Arg, Gln, Ser or Lys); Xaa at res.12 = (Asp or Glu); Xaa at res.13 = (Leu or Val); Xaa at res.16 = (Gln, Leu, Asp, His or Asn); Xaa at res.17 = (Asp, Arg, or Asn); Xaa at res.19 = (Ile or Val); Xaa at res.20 = (Ile or Val); Xaa at res.23 = (Glu, Gln, Leu, Lys, Pro or Arg); Xaa at res.25 = (Tyr or Phe); Xaa at res.26 = (Ala, Ser, Asp, Met, His, Leu, or Gln); Xaa at res.28 = (Tyr, Asn or Phe); Xaa at res.31 = (Glu, His, Tyr, Asp or Gln); Xaa at res.33 = Glu, Lys, Asp or Gln); Xaa at res.35 = (Ala, Ser or Pro); Xaa at res.36 = (Phe, Leu or Tyr); Xaa at res.38 = (Leu or Val); Xaa at res.39 = (Asn, Asp, Ala or Thr); Xaa at res.40 = (Ser, Asp, Glu, Leu or Ala); Xaa at res.41 = (Tyr, Cys, His, Ser or Ile); Xaa at res.42 = (Met, Phe, Gly or Leu); Xaa at res.44 = (Ala, Ser or Gly); Xaa at res.45 = (Thr, Leu or Ser); Xaa at res.49 = (Ile or Val); Xaa at res.50 = (Val or Leu); Xaa at res.51 = (Gln or Arg); Xaa at res.52 = (Thr, Ala or Ser); Xaa at res.54 = (Val or Met); Xaa at res.55 = (His or Asn); Xaa at res.56 = (Phe, Leu, Asn, Ser, Ala or Val); Xaa at res.57 = (Ile, Met, Asn, Ala or Val); Xaa at res.58 = (Asn, Lys, Ala or Glu); Xaa at res.59 = (Pro or Ser); Xaa at res.60 = (Glu, Asp, or Gly); Xaa at res.61 = (Thr, Ala, Val, Lys, Asp, Tyr, Ser or Ala); Xaa at res.62 = (Val, Ala or Ile); Xaa at res.63 = (Pro or Asp); Xaa at res.64 = (Lys or Leu); Xaa at res.65 = (Pro or Ala); Xaa at res.68 = (Ala or Val); Xaa at res.70 = (Thr or Ala); Xaa at res.71 = (Gln, Lys, Arg or Glu); Xaa at res.72 = (Leu, Met or Val); Xaa at res.73 = (Asn, Ser or Asp);

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Xaa at res.74 = (Ala, Pro or Ser); Xaa at res.75 =
(Ile, Thr or Val); Xaa at res.76 = (Ser or Ala); Xaa at
res.77 = (Val or Met); Xaa at res.79 = (Tyr or Phe);
Xaa at res.80 = (Phe, Tyr or Leu); Xaa at res.81 = (Asp
5 or Asn); Xaa at res.82 = (Asp, Glu, Asn or Ser); Xaa at
res.83 = (Ser, Gln, Asn or Tyr); Xaa at res.84 = (Ser,
Asn, Asp or Glu); Xaa at res.85 = (Asn, Thr or Lys);
Xaa at res.87 = (Ile or Val); Xaa at res.89 = (Lys or
Arg); Xaa at res.90 = (Lys, Asn, Gln or His); Xaa at
10 res.91 = (Tyr or His); Xaa at res.92 = (Arg, Gln or
Glu); Xaa at res.93 = (Asn, Glu or Asp); Xaa at res.95
= (Val, Thr or Ala); Xaa at res.97 = (Arg, Lys, Val,
Asp or Glu); Xaa at res.98 = (Ala, Gly or Glu); and Xaa
at res.102 = (His or Arg).

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Similarly, Generic Sequence 5 (Seq. ID No. 30) and
Generic Sequence 6 (Seq. ID No. 31) accommodate the
homologies shared among all the morphogen protein
family members identified in Table II. Specifically,
20 Generic Sequences 5 and 6 are composite amino acid
sequences of human OP-1 (hOP-1, Seq. ID Nos. 5 and 16-
17), mouse OP-1 (mOP-1, Seq. ID Nos. 6 and 18-19),
human and mouse OP-2 (Seq. ID Nos. 7, 8, and 20-22),
CBMP2A (Seq. ID No. 9), CBMP2B (Seq. ID No. 10), DPP
25 (from Drosophila, Seq. ID No. 11), Vgl, (from Xenopus,
Seq. ID No. 12), Vgr-1 (from mouse, Seq. ID No. 13),
and GDF-1 (from mouse, Seq. ID No. 14), human BMP3
(Seq. ID No. 26), human BMP5 (Seq. ID No. 27), human
BMP6 (Seq. ID No. 28) and 60(A) (from Drosophila, Seq.
30 ID Nos. 24-25). The generic sequences include both the
amino acid identity shared by these sequences in the
C-terminal domain, defined by the six and seven
cysteine skeltons (Generic Sequences 5 and 6,
respectively), as well as alternative residues for the
35 variable positions within the sequence. As for Generic

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Xaa Xaa Xaa Leu Xaa Xaa Xaa

70

75

Xaa Xaa Xaa Xaa Val Xaa Leu Xaa

80

5 Xaa Xaa Xaa Xaa Met Xaa Val Xaa

85

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Xaa Cys Xaa Cys Xaa

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wherein each Xaa is independently selected from a group
 10 of one or more specified amino acids defined as
 follows: "Res." means "residue" and Xaa at res.2 =
 (Tyr or Lys); Xaa at res.3 = Val or Ile); Xaa at res.4
 = (Ser, Asp or Glu); Xaa at res.6 = (Arg, Gln, Ser, Lys
 or Ala); Xaa at res.7 = (Asp, Glu or Lys); Xaa at res.8
 15 = (Leu, Val or Ile); Xaa at res.11 = (Gln, Leu, Asp,
 His, Asn or Ser); Xaa at res.12 = (Asp, Arg, Asn or
 Glu); Xaa at res.14 = (Ile or Val); Xaa at res.15 =
 (Ile or Val); Xaa at res.16 (Ala or Ser); Xaa at res.18
 = (Glu, Gln, Leu, Lys, Pro or Arg); Xaa at res.19 =
 20 (Gly or Ser); Xaa at res.20 = (Tyr or Phe); Xaa at
 res.21 = (Ala, Ser, Asp, Met, His, Gln, Leu or Gly);
 Xaa at res.23 = (Tyr, Asn or Phe); Xaa at res.26 =
 (Glu, His, Tyr, Asp, Gln or Ser); Xaa at res.28 = (Glu,
 Lys, Asp, Gln or Ala); Xaa at res.30 = (Ala, Ser, Pro,
 25 Gln or Asn); Xaa at res.31 = (Phe, Leu or Tyr); Xaa at
 res.33 = (Leu, Val or Met); Xaa at res.34 = (Asn, Asp,
 Ala, Thr or Pro); Xaa at res.35 = (Ser, Asp, Glu, Leu,
 Ala or Lys); Xaa at res.36 = (Tyr, Cys, His, Ser or
 Ile); Xaa at res.37 = (Met, Phe, Gly or Leu); Xaa at
 30 res.38 = (Asn, Ser or Lys); Xaa at res.39 = (Ala, Ser,
 Gly or Pro); Xaa at res.40 = (Thr, Leu or Ser); Xaa at
 res.44 = (Ile, Val or Thr); Xaa at res.45 = (Val, Leu

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or Ile); Xaa at res.46 = (Gln or Arg); Xaa at res.47 =
(Thr, Ala or Ser); Xaa at res.48 = (Leu or Ile); Xaa at
res.49 = (Val or Met); Xaa at res.50 = (His, Asn or
Arg); Xaa at res.51 = (Phe, Leu, Asn, Ser, Ala or Val);
5 Xaa at res.52 = (Ile, Met, Asn, Ala, Val or Leu); Xaa
at res.53 = (Asn, Lys, Ala, Glu, Gly or Phe); Xaa at
res.54 = (Pro, Ser or Val); Xaa at res.55 = (Glu, Asp,
Asn, Gly, Val or Lys); Xaa at res.56 = (Thr, Ala, Val,
Lys, Asp, Tyr, Ser, Ala, Pro or His); Xaa at res.57 =
10 (Val, Ala or Ile); Xaa at res.58 = (Pro or Asp); Xaa at
res.59 = (Lys, Leu or Glu); Xaa at res.60 = (Pro or
Ala); Xaa at res.63 = (Ala or Val); Xaa at res.65 =
(Thr, Ala or Glu); Xaa at res.66 = (Gln, Lys, Arg or
Glu); Xaa at res.67 = (Leu, Met or Val); Xaa at res.68
15 = (Asn, Ser, Asp or Gly); Xaa at res.69 = (Ala, Pro or
Ser); Xaa at res.70 = (Ile, Thr, Val or Leu); Xaa at
res.71 = (Ser, Ala or Pro); Xaa at res.72 = (Val, Met
or Ile); Xaa at res.74 = (Tyr or Phe); Xaa at res.75 =
(Phe, Tyr, Leu or His); Xaa at res.76 = (Asp, Asn or
20 Leu); Xaa at res.77 = (Asp, Glu, Asn or Ser); Xaa at
res.78 = (Ser, Gln, Asn, Tyr or Asp); Xaa at res.79 =
(Ser, Asn, Asp, Glu or Lys); Xaa at res.80 = (Asn, Thr
or Lys); Xaa at res.82 = (Ile, Val or Asn); Xaa at
res.84 = (Lys or Arg); Xaa at res.85 = (Lys, Asn, Gln,
25 His or Val); Xaa at res.86 = (Tyr or His); Xaa at
res.87 = (Arg, Gln, Glu or Pro); Xaa at res.88 = (Asn,
Glu or Asp); Xaa at res.90 = (Val, Thr, Ala or Ile);
Xaa at res.92 = (Arg, Lys, Val, Asp or Glu); Xaa at
res.93 = (Ala, Gly, Glu or Ser); Xaa at res.95 = (Gly
30 or Ala) and Xaa at res.97 = (His or Arg).

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Generic Sequence 6

	Cys	Xaa	Xaa	Xaa	Xaa	Leu	Xaa	Xaa	Xaa	Phe
	1					5				10
5	Xaa	Xaa	Xaa	Gly	Trp	Xaa	Xaa	Trp	Xaa	
						15				
	Xaa	Xaa	Pro	Xaa	Xaa	Xaa	Xaa	Ala		
	20					25				
	Xaa	Tyr	Cys	Xaa	Gly	Xaa	Cys	Xaa		
10			30					35		
	Xaa	Pro	Xaa	Xaa	Xaa	Xaa	Xaa			
						40				
	Xaa	Xaa	Xaa	Asn	His	Ala	Xaa	Xaa		
			45					50		
15	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa		
						55				
	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Cys		
			60					65		
	Cys	Xaa	Pro	Xaa	Xaa	Xaa	Xaa	Xaa		
20						70				
	Xaa	Xaa	Xaa	Leu	Xaa	Xaa	Xaa			
			75					80		
	Xaa	Xaa	Xaa	Xaa	Val	Xaa	Leu	Xaa		
						85				
25	Xaa	Xaa	Xaa	Xaa	Met	Xaa	Val	Xaa		
			90					95		
	Xaa	Cys	Xaa	Cys	Xaa					
						100				

30 wherein each Xaa is independently selected from a group
of one or more specified amino acids as defined by the
following: "Res." means "residue" and Xaa at res.2 =
(Lys, Arg, Ala or Gln); Xaa at res.3 = (Lys, Arg or
Met); Xaa at res.4 = (His, Arg or Gln); Xaa at res.5 =
35 (Glu, Ser, His, Gly, Arg, Pro, Thr, or Tyr); Xaa at

res.7 = (Tyr or Lys); Xaa at res.8 = (Val or Ile); Xaa
at res.9 = (Ser, Asp or Glu); Xaa at res.11 = (Arg,
Gln, Ser, Lys or Ala); Xaa at res.12 = (Asp, Glu, or
Lys); Xaa at res.13 = (Leu, Val or Ile); Xaa at res.16
5 = (Gln, Leu, Asp, His, Asn or Ser); Xaa at res.17 =
(Asp, Arg, Asn or Glu); Xaa at res.19 = (Ile or Val);
Xaa at res.20 = (Ile or Val); Xaa at res.21 = (Ala or
Ser); Xaa at res.23 = (Glu, Gln, Leu, Lys, Pro or Arg);
Xaa at res.24 = (Gly or Ser); Xaa at res.25 = (Tyr or
10 Phe); Xaa at res.26 = (Ala, Ser, Asp, Met, His, Gln,
Leu, or Gly); Xaa at res.28 = (Tyr, Asn or Phe); Xaa at
res.31 = (Glu, His, Tyr, Asp, Gln or Ser); Xaa at
res.33 = Glu, Lys, Asp, Gln or Ala); Xaa at res.35 =
(Ala, Ser, Pro, Gln or Asn); Xaa at res.36 = (Phe, Leu
15 or Tyr); Xaa at res.38 = (Leu, Val or Met); Xaa at
res.39 = (Asn, Asp, Ala, Thr or Pro); Xaa at res.40 =
(Ser, Asp, Glu, Leu, Ala or Lys); Xaa at res.41 = (Tyr,
Cys, His, Ser or Ile); Xaa at res.42 = (Met, Phe, Gly
or Leu); Xaa at res.43 = (Asn, Ser or Lys); Xaa at
20 res.44 = (Ala, Ser, Gly or Pro); Xaa at res.45 = (Thr,
Leu or Ser); Xaa at res.49 = (Ile, Val or Thr); Xaa at
res.50 = (Val, Leu or Ile); Xaa at res.51 = (Gln or
Arg); Xaa at res.52 = (Thr, Ala or Ser); Xaa at res.53
= (Leu or Ile); Xaa at res.54 = (Val or Met); Xaa at
25 res.55 = (His, Asn or Arg); Xaa at res.56 = (Phe, Leu,
Asn, Ser, Ala or Val); Xaa at res.57 = (Ile, Met, Asn,
Ala, Val or Leu); Xaa at res.58 = (Asn, Lys, Ala, Glu,
Gly or Phe); Xaa at res.59 = (Pro, Ser or Val); Xaa at
res.60 = (Glu, Asp, Gly, Val or Lys); Xaa at res.61 =
30 (Thr, Ala, Val, Lys, Asp, Tyr, Ser, Ala, Pro or His);
Xaa at res.62 = (Val, Ala or Ile); Xaa at res.63 = (Pro
or Asp); Xaa at res.64 = (Lys, Leu or Glu); Xaa at
res.65 = (Pro or Ala); Xaa at res.68 = (Ala or Val);
Xaa at res.70 = (Thr, Ala or Glu); Xaa at res.71 =
35 (Gln, Lys, Arg or Glu); Xaa at res.72 = (Leu, Met or

Val); Xaa at res.73 = (Asn, Ser, Asp or Gly); Xaa at
res.74 = (Ala, Pro or Ser); Xaa at res.75 = (Ile, Thr,
Val or Leu); Xaa at res.76 = (Ser, Ala or Pro); Xaa at
res.77 = (Val, Met or Ile); Xaa at res.79 = (Tyr or
5 Phe); Xaa at res.80 = (Phe, Tyr, Leu or His); Xaa at
res.81 = (Asp, Asn or Leu); Xaa at res.82 = (Asp, Glu,
Asn or Ser); Xaa at res.83 = (Ser, Gln, Asn, Tyr or
Asp); Xaa at res.84 = (Ser, Asn, Asp, Glu or Lys); Xaa
at res.85 = (Asn, Thr or Lys); Xaa at res.87 = (Ile,
10 Val or Asn); Xaa at res.89 = (Lys or Arg); Xaa at
res.90 = (Lys, Asn, Gln, His or Val); Xaa at res.91 =
(Tyr or His); Xaa at res.92 = (Arg, Gln, Glu or Pro);
Xaa at res.93 = (Asn, Glu or Asp); Xaa at res.95 =
(Val, Thr, Ala or Ile); Xaa at res.97 = (Arg, Lys, Val,
15 Asp or Glu); Xaa at res.98 = (Ala, Gly, Glu or Ser);
Xaa at res.100 = (Gly or Ala); and Xaa at res.102 =
(His or Arg).

Particularly useful sequences for use as
20 morphogens in this invention include the C-terminal
domains, e.g., the C-terminal 96-102 amino acid
residues of Vgl, Vgr-1, DPP, OP-1, OP-2, CBMP-2A,
CBMP-2B, GDF-1 (see Table II, below, and Seq. ID
Nos. 5-14), as well as proteins comprising the
25 C-terminal domains of 60A, BMP3, BMP5 and BMP6 (see
Seq. ID Nos. 24-28), all of which include at least the
conserved six or seven cysteine skeleton. In addition,
biosynthetic constructs designed from the generic
sequences, such as COP-1, 3-5, 7, 16, disclosed in U.S.
30 Pat. No. 5,011,691, also are useful. Other sequences
include the inhibins/activin proteins (see, for
example, U.S. Pat. Nos. 4,968,590 and 5,011,691).
Accordingly, other useful sequences are those sharing
at least 70% amino acid sequence homology or
35 "similarity", and preferably 80% homology or similarity

with any of the sequences above. These are anticipated to include allelic and species variants and mutants, and biosynthetic muteins, as well as novel members of this morphogenic family of proteins. Particularly -
5 envisioned in the family of related proteins are those proteins exhibiting morphogenic activity and wherein the amino acid changes from the preferred sequences include conservative changes, e.g., those as defined by Dayoff et al., Atlas of Protein Sequence and Structure;
10 vol. 5, Suppl. 3, pp. 345-362, (M.O. Dayoff, ed., Nat'l BioMed. Research Fdn., Washington, D.C. 1979). As used herein, potentially useful sequences are aligned with a known morphogen sequence using the method of Needleman et al. ((1970) J.Mol.Biol. 48:443-453) and identities
15 calculated by the Align program (DNASTar, Inc.). "Homology" or "similarity" as used herein includes allowed conservative changes as defined by Dayoff et al.

20 The currently most preferred protein sequences useful as morphogens in this invention include those having greater than 60% identity, preferably greater than 65% identity, with the amino acid sequence defining the conserved six cysteine skeleton of hOP1
25 (e.g., residues 43-139 of Seq. ID No. 5). These most preferred sequences include both allelic and species variants of the OP-1 and OP-2 proteins, including the Drosophila 60A protein. Accordingly, in another preferred aspect of the invention, useful morphogens
30 include active proteins comprising species of polypeptide chains having the generic amino acid sequence herein referred to as "OPX", which accommodates the homologies between the various identified species of OP1 and OP2 (Seq. ID No. 29).

The morphogens useful in the methods, composition and devices of this invention include proteins comprising any of the polypeptide chains described above, whether isolated from naturally-occurring
5 sources, or produced by recombinant DNA or other synthetic techniques, and includes allelic and species variants of these proteins, naturally-occurring or biosynthetic mutants thereof, as well as various truncated and fusion constructs. Deletion or addition
10 mutants also are envisioned to be active, including those which may alter the conserved C-terminal cysteine skeleton, provided that the alteration does not functionally disrupt the relationship of these cysteines in the folded structure. Accordingly, such
15 active forms are considered the equivalent of the specifically described constructs disclosed herein. The proteins may include forms having varying glycosylation patterns, varying N-termini, a family of related proteins having regions of amino acid sequence
20 homology, and active truncated or mutated forms of native or biosynthetic proteins, produced by expression of recombinant DNA in host cells.

The morphogenic proteins can be expressed from
25 intact or truncated cDNA or from synthetic DNAs in procaryotic or eucaryotic host cells, and purified, cleaved, refolded, and dimerized to form morphogenically active compositions. Currently preferred host cells include E. coli or mammalian
30 cells, such as CHO, COS or BSC cells. A detailed description of the morphogens useful in the methods,

5

10

20

Brief Description of the Drawings:

The foregoing and other objects and features of this invention, as well as the invention itself, may be more fully understood from the following description, when read together with the accompanying drawings, in which:

Fig. 1(A and B) are photomicrographs illustrating the ability of morphogen (OP-1) to induce transformed neuroblastoma x glioma cells (1A) to redifferentiate to a morphology characteristic of untransformed neurons (1B);

Fig. 2A is a dose response curve for the induction of the 180 kDa and 140 kDa N-CAM isoforms in morphogen-treated NG108-15 cells;

Fig. 2B is a photomicrograph of a Western blot of whole cell extracts from morphogen-treated NG108-15 cells with an N-CAM-specific antibody; and

Fig. 3 is a plot of the mean number of cell aggregates counted in 20 randomly selected fields as a function of morphogen concentration.

Fig. 4 is a photomicrograph of an immunoblot demonstrating the presence of OP-1 in human serum.

Detailed Description of the Invention

It now has been discovered that the proteins described herein are effective agents for enhancing the survival of neurons, particularly neurons at risk of dying, and for maintaining neural pathways in a mammal. As described herein, these proteins ("morphogens") are capable of enhancing survival of non-mitotic neurons, stimulating neuronal CAM expression, maintaining the phenotypic expression of differentiated neurons, inducing the redifferentiation of transformed cells of neural origin, and stimulating axonal growth over breaks in neural processes, particularly large gaps in distal axons. The proteins also are capable of providing a neuroprotective effect to alleviate the tissue destructive effects associated with immunologically-related nerve tissue damage. Finally, the proteins may be used as part of a method for monitoring the viability of nerve tissue in a mammal.

Provided below are detailed descriptions of suitable morphogens useful in the methods, compositions and devices of this invention, as well as methods for their administration and application, and numerous, nonlimiting examples which 1) illustrate the suitability of the morphogens and morphogen-stimulating agents described herein as therapeutic agents for maintaining neural pathways in a mammal and enhancing survival of neuronal cells at risk of dying; and 2) provide assays with which to test candidate morphogens and morphogen-stimulating agents for their efficacy.

I. Useful Morphogens

As defined herein a protein is morphogenic if it is capable of inducing the developmental cascade of cellular and molecular events that culminate in the formation of new, organ-specific tissue and comprises at least the conserved C-terminal six cysteine skeleton or its functional equivalent (see supra).

Specifically, the morphogens generally are capable of all of the following biological functions in a morphogenically permissive environment: stimulating proliferation of progenitor cells; stimulating the differentiation of progenitor cells; stimulating the proliferation of differentiated cells; and supporting the growth and maintenance of differentiated cells, including the "redifferentiation" of transformed cells. Details of how the morphogens useful in the method of this invention first were identified, as well as a description on how to make, use and test them for morphogenic activity are disclosed in USSN 667,274, filed March 11, 1991 and USSN 752,764, filed August 30, 1991, the disclosures of which are hereby incorporated by reference. As disclosed therein, the morphogens may be purified from naturally-sourced material or recombinantly produced from procaryotic or eucaryotic host cells, using the genetic sequences disclosed therein. Alternatively, novel morphogenic sequences may be identified following the procedures disclosed therein.

30

Particularly useful proteins include those which comprise the naturally derived sequences disclosed in Table II. Other useful sequences include biosynthetic

constructs such as those disclosed in U.S. Pat.
5,011,691, the disclosure of which is incorporated
herein by reference (e.g., COP-1, COP-3, COP-4, COP-5,
COP-7, and COP-16).

5

Accordingly, the morphogens useful in the methods
and compositions of this invention also may be
described by morphogenically active proteins having
amino acid sequences sharing 70% or, preferably, 80%
10 homology (similarity) with any of the sequences
described above, where "homology" is as defined herein
above.

The morphogens useful in the method of this
15 invention also can be described by any of the 6 generic
sequences described herein (Generic Sequences 1, 2, 3,
4, 5 and 6). Generic sequences 1 and 2 also may
include, at their N-terminus, the sequence

20 Cys Xaa Xaa Xaa Xaa (Seq. ID No. 15)
1 5

Table II, set forth below, compares the amino acid
sequences of the active regions of native proteins that
25 have been identified as morphogens, including human
OP-1 (hOP-1, Seq. ID Nos. 5 and 16-17), mouse OP-1
(mOP-1, Seq. ID Nos. 6 and 18-19), human and mouse OP-2
(Seq. ID Nos. 7, 8, and 20-23), CBMP2A (Seq. ID No. 9),
CBMP2B (Seq. ID No. 10), BMP3 (Seq. ID No. 26), DPP
30 (from Drosophila, Seq. ID No. 11), Vgl, (from Xenopus,
Seq. ID No. 12), Vgr-1 (from mouse, Seq. ID No. 13),
GDF-1 (from mouse, Seq. ID Nos. 14, 32 and 33), 60A
protein (from Drosophila, Seq. ID Nos. 24 and 25), BMP5
(Seq. ID No. 27) and BMP6 (Seq. ID No. 28). The
35 sequences are aligned essentially following the method

5 Three dashes indicates that no amino acid is present in that position, and are included for purposes of illustrating homologies. For example, amino acid residue 60 of CBMP-2A and CBMP-2B is "missing". Of course, both these amino acid sequences in this region
0 comprise Asn-Ser (residues 58, 59), with CBMP-2A then comprising Lys and Ile, whereas CBMP-2B comprises Ser and Ile.

15

[illegible]

5	hOP-2	Gln	Leu	...
	mOP-2	Ser	Leu	...
	DPP	Asp	...	Ser	...	Val	Asp	...
	Vgl	Glu	...	Lys	...	Val	Asn
	Vgr-1	Gln	...	Val
	CBMP-2A	Asp	...	Ser	...	Val	Asn	...
	CBMP-2B	Asp	...	Ser	...	Val	Asn	...
	BMP3	Asp	...	Ala	...	Ile	Ser	Glu
	GDF-1	Glu	Val	His	Arg
	60A	Asp	...	Lys	His	...
10	BMP5
	BMP6	Gln
			10					15		
20	hOP-1	Trp	Ile	Ile	Ala	Pro	Glu	Gly	Tyr	Ala
	mOP-1
	hOP-2	...	Val	Gln	Ser
	mOP-2	...	Val	Gln	Ser
	DPP	Val	Leu	Asp
	Vgl	...	Val	Gln	Met
	Vgr-1	Lys
	CBMP-2A	Val	Pro	His
	CBMP-2B	Val	Pro	Gln
	BMP3	Ser	...	Lys	Ser	Phe	Asp
25	GDF-1	...	Val	Arg	...	Phe	Leu
	60A	Gly
	BMP5
	BMP6	Lys
				20					25	
30										
35	hOP-1	Ala	Tyr	Tyr	Cys	Glu	Gly	Glu	Cys	Ala
	mOP-1
	hOP-2	Ser
	mOP-2

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[illegible]

	CBMP-2A
	CBMP-2B
	BMP3	Ser	Thr	Ile	...	Ser	Ile
	GDF-1	Leu	Val	Leu	Arg	Ala	...
5	60A
	BMP5
	BMP6
		45					50			
10	hOP-1	Val	His	Phe	Ile	Asn	Pro	Glu	Thr	Val
	mOP-1	Asp
	hOP-2	...	His	Leu	Met	Lys	...	Asn	Ala	...
	mOP-2	...	His	Leu	Met	Lys	...	Asp	Val	...
15	DPP	...	Asn	Asn	Asn	Gly	Lys	...
	Vgl	Ser	...	Glu	Asp	Ile
	Vgr-1	Val	Met	Tyr	...
	CBMP-2A	...	Asn	Ser	Val	...	Ser	---	Lys	Ile
	CBMP-2B	...	Asn	Ser	Val	...	Ser	---	Ser	Ile
20	BMP3	...	Arg	Ala**	Gly	Val	Val	Pro	Gly	Ile
	GDF-1	Met	...	Ala	Ala	Ala	...	Gly	Ala	Ala
	60A	Leu	Leu	Glu	...	Lys	Lys	...
	BMP5	Leu	Met	Phe	...	Asp	His	...
	BMP6	Leu	Met	Tyr	...
25			55					60		
	hOP-1	Pro	Lys	Pro	Cys	Cys	Ala	Pro	Thr	Gln
	mOP-1
30	hOP-2	Ala	Lys
	mOP-2	Ala	Lys
	DPP	Ala	Val
	Vgl	...	Leu	Val	Lys
	Vgr-1	Lys
35	CBMP-2A	Ala	Val	Glu

5	CBMP-2B	Ala	Val	Glu
	BMP3	...	Glu	Val	...	Glu	Lys
	GDF-1	Asp	Leu	Val	...	Ala	Arg
	60A	Arg
	BMP5	Lys
	BMP6	Lys
				65	70					
10	hOP-1	Leu	Asn	Ala	Ile	Ser	Val	Leu	Tyr	Phe
	mOP-1
	hOP-2	...	Ser	...	Thr	Tyr
	mOP-2	...	Ser	...	Thr	Tyr
15	Vgl	Met	Ser	Pro	Met	...	Phe	Tyr
	Vgr-1	Val
	DPP	...	Asp	Ser	Val	Ala	Met	Leu
	CBMP-2A	...	Ser	Met	Leu
	CBMP-2B	...	Ser	Met	Leu
	BMP3	Met	Ser	Ser	Leu	...	Ile	...	Phe	Tyr
20	GDF-1	...	Ser	Pro	Phe	...
	60A	...	Gly	...	Leu	Pro	His
	BMP5
	BMP6
				75	80					
25	hOP-1	Asp	Asp	Ser	Ser	Asn	Val	Ile	Leu	Lys
	mOP-1
	hOP-2	...	Ser	...	Asn	Arg
	mOP-2	...	Ser	...	Asn	Arg
30	DPP	Asn	...	Gln	...	Thr	...	Val
	Vgl	...	Asn	Asn	Asp	Val	...	Arg
	Vgr-1	Asn
	CBMP-2A	...	Glu	Asn	Glu	Lys	...	Val
35	CBMP-2B	...	Glu	Tyr	Asp	Lys	...	Val
	BMP3	...	Glu	Asn	Lys	Val
	GDF-1	...	Asn	...	Asp	Val	...	Arg

	60A	Leu	Asn	Asp	Glu	Asn
	BMP5
	BMP6	Asn
						85				
5										
	hOP-1	Lys	Tyr	Arg	Asn	Met	Val	Val	Arg	
	mOP-1	
	hOP-2	...	His	Lys	
10	mOP-2	...	His	Lys	
	DPP	Asn	...	Gln	Glu	...	Thr	...	Val	
	Vgl	His	...	Glu	Ala	...	Asp	
	Vgr-1	
	CBMP-2A	Asn	...	Gln	Asp	Glu	
15	CBMP-2B	Asn	...	Gln	Glu	Glu	
	BMP3	Val	...	Pro	Thr	...	Glu	
	GDF-1	Gln	...	Glu	Asp	Asp	
	60A	Ile	...	Lys	
	BMP5	
20	BMP6	Trp	
		90					95			
	hOP-1	Ala	Cys	Gly	Cys	His				
25	mOP-1				
	hOP-2				
	mOP-2				
	DPP	Gly	Arg				
	Vgl	Glu	Arg				
30	Vgr-1				
	CBMP-2A	Gly	Arg				
	CBMP-2B	Gly	Arg				

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	BMP3	Ser	...	Ala	...	Arg
	GDF-1	Glu	Arg
	60A	Ser
	BMP5	Ser
5	BMP6
				100		

**Between residues 56 and 57 of BMP3 is a Val residue;
between residues 43 and 44 of GDF-1 lies
the amino acid sequence Gly-Gly-Pro-Pro.

10

As is apparent from the foregoing amino acid
sequence comparisons, significant amino acid changes
can be made within the generic sequences while
15 retaining the morphogenic activity. For example, while
the GDF-1 protein sequence depicted in Table II shares
only about 50% amino acid identity with the hOP1
sequence described therein, the GDF-1 sequence shares
greater than 70% amino acid sequence homology (or
20 "similarity") with the hOP1 sequence, where "homology"
or "similarity" includes allowed conservative amino
acid changes within the sequence as defined by Dayoff,
et al., Atlas of Protein Sequence and Structure vol.5,
supp.3, pp.345-362, (M.O. Dayoff, ed., Nat'l BioMed.
25 Res. Fd'n, Washington D.C. 1979.)

The currently most preferred protein sequences
useful as morphogens in this invention include those
having greater than 60% identity, preferably greater
30 than 65% identity, with the amino acid sequence
defining the conserved six cysteine skeleton of hOP1
(e.g., residues 43-139 of Seq. ID No. 5). These most
preferred sequences include both allelic and species
variants of the OP-1 and OP-2 proteins, including the
35 *Drosophila* 60A protein. Accordingly, in still another

preferred aspect, the invention includes morphogens comprising species of polypeptide chains having the generic amino acid sequence referred to herein as "OPX", which defines the seven cysteine skeleton and accommodates the identities between the various identified mouse and human OP1 and OP2 proteins. OPX is presented in Seq. ID No. 29. As described therein, each Xaa at a given position independently is selected from the residues occurring at the corresponding position in the C-terminal sequence of mouse or human OP1 or OP2 (see Seq. ID Nos. 5-8 and/or Seq. ID Nos. 16-23).

II. Formulations and Methods for Administering
Therapeutic Agents

The morphogens may be provided to an individual by any suitable means, preferably directly (e.g., locally, as by injection to a nerve tissue locus) or systemically (e.g., parenterally or orally). Where the morphogen is to be provided parenterally, such as by intravenous, subcutaneous, intramuscular, intraorbital, ophthalmic, intraventricular, intracranial, intracapsular, intraspinal, intracisternal, intraperitoneal, buccal, rectal, vaginal, intranasal or by aerosol administration, the morphogen preferably comprises part of an aqueous solution. The solution is physiologically acceptable so that in addition to delivery of the desired morphogen to the patient, the solution does not otherwise adversely affect the patient's electrolyte and volume balance. The aqueous medium for the morphogen thus may comprise normal physiologic saline (9.85% NaCl, 0.15M), pH 7-7.4. The aqueous solution containing the morphogen can be made, for example, by dissolving the protein in 50% ethanol

containing acetonitrile in 0.1% trifluoroacetic acid (TFA) or 0.1% HCl, or equivalent solvents. One volume of the resultant solution then is added, for example, to ten volumes of phosphate buffered saline (PBS),
5 which further may include 0.1-0.2% human serum albumin (HSA). The resultant solution preferably is vortexed extensively. If desired, a given morphogen may be made more soluble by association with a suitable molecule. For example, association of the mature dimer with the
10 pro domain of the morphogen increases solubility of the protein significantly. In fact, the endogenous protein is thought to be transported in this form. Another molecule capable of enhancing solubility and particularly useful for oral administrations, is
15 casein. For example, addition of 0.2% casein increases solubility of the mature active form of OP-1 by 80%. Other components found in milk and/or various serum proteins also may be useful.

20 Useful solutions for parenteral administration may be prepared by any of the methods well known in the pharmaceutical art, described, for example, in Remington's Pharmaceutical Sciences (Gennaro, A., ed.), Mack Pub., 1990. Formulations may include, for
25 example, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, hydrogenated naphthalenes, and the like. Formulations for direct administration, in particular, may include glycerol and other compositions of high viscosity. Biocompatible,
30 preferably bioresorbable, polymers, including, for example, hyaluronic acid, collagen, polybutyrate, tricalcium phosphate, lactide and lactide/glycolide copolymers, may be useful excipients to control the release of the morphogen in vivo. Other potentially
35 useful parenteral delivery systems for these morphogens

include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. Formulations for inhalation administration contain as excipients, for example, lactose, or may be aqueous solutions containing, for example, polyoxyethylene-9-lauryl ether, glycocholate and deoxycholate, or oily solutions for administration in the form of nasal drops, or as a gel to be applied intranasally. Formulations for parenteral administration may also include glycocholate for buccal administration, methoxysalicylate for rectal administration, or cutric acid for vaginal administration.

Alternatively, the morphogens described herein may be administered orally. Oral administration of proteins as therapeutics generally is not practiced as most proteins are readily degraded by digestive enzymes and acids in the mammalian digestive system before they can be absorbed into the bloodstream. However, the morphogens described herein typically are acid stable and protease-resistant (see, for example, U.S. Pat.No. 4,968,590.) In addition, at least one morphogen, OP-1, has been identified in mammary gland extract, colostrum and 57-day milk. Moreover, the OP-1 purified from mammary gland extract is morphogenically active. Specifically, this protein induces endochondral bone formation in mammals when implanted subcutaneously in association with a suitable matrix material, using a standard in vivo bone assay, such as is disclosed in U.S. Pat.No. 4,968,590. Moreover, the morphogen also is detected in the bloodstream (see Example 9, below). Finally, soluble form morphogen, e.g., mature morphogen associated with the pro domain, is capable of maintaining neural pathways in a mammal (See Examples 4

and 6 below). These findings indicate that oral and parenteral administration are viable means for administering morphogens to an individual. In addition, while the mature forms of certain morphogens described herein typically are sparingly soluble, the morphogen form found in milk (and mammary gland extract and colostrum) is readily soluble, probably by association of the mature, morphogenically active form with part or all of the pro domain of the intact sequence and/or by association with one or more milk components. Accordingly, the compounds provided herein also may be associated with molecules capable of enhancing their solubility in vitro or in vivo.

The compounds provided herein also may be associated with molecules capable of targeting the morphogen or morphogen-stimulating agent to nerve tissue. For example, an antibody, antibody fragment, or other binding protein that interacts specifically with a surface molecule on nerve tissue cells, including neuronal or glial cells, may be used. Useful targeting molecules may be designed, for example, using the single chain binding site technology disclosed, for example, in U.S. Pat. No. 5,091,513.

As described above, the morphogens provided herein share significant sequence homology in the C-terminal active domains. By contrast, the sequences typically diverge significantly in the sequences which define the pro domain. Accordingly, the pro domain is thought to be morphogen-specific. As described above, it is also known that the various morphogens identified to date are differentially expressed in the different tissues. Accordingly, without being limited to any given theory, it is likely that, under natural conditions in the

body, selected morphogens typically act on a given tissue. Accordingly, part or all of the pro domains which have been identified associated with the active form of the morphogen in solution, may serve as
5 targeting molecules for the morphogens described herein. For example, the pro domains may interact specifically with one or more molecules at the target tissue to direct the morphogen associated with the pro domain to that tissue. Accordingly, another useful
10 targeting molecule for targeting morphogen to nerve tissue is part or all of a morphogen pro domain, particularly part or all of the pro domains of OP-1 or GDF-1, both of which proteins are found naturally associated with nerve tissue.

15 Finally, the morphogens or morphogen-stimulating agents provided herein may be administered alone or in combination with other molecules known to be beneficial in maintaining neural pathways, including nerve growth
20 factors and anti-inflammatory agents.

The compounds provided herein can be formulated into pharmaceutical compositions by admixture with pharmaceutically acceptable nontoxic excipients and
25 carriers. As noted above, such compositions may be prepared for parenteral administration, particularly in the form of liquid solutions or suspensions; for oral administration, particularly in the form of tablets or capsules; or intranasally, particularly in the form of
30 powders, nasal drops, or aerosols.

The compositions can be formulated for parenteral or oral administration to humans or other mammals in therapeutically effective amounts, e.g., amounts which
35 provide appropriate concentrations for a time

sufficient to eliminate or reduce the patient's pathological condition, to provide therapy for the neurological diseases and disorders described above, and amounts effective to enhance neural cell survival
5 an/or to protect neurons and neural pathways in anticipation of injury to nerve tissue.

As will be appreciated by those skilled in the art, the concentration of the compounds described in a
10 therapeutic composition will vary depending upon a number of factors, including the dosage of the drug to be administered, the chemical characteristics (e.g., hydrophobicity) of the compounds employed, and the route of administration. The preferred dosage of drug
15 to be administered also is likely to depend on such variables as the type and extent of progression of the neurological disease, the overall health status of the particular patient, the relative biological efficacy of the compound selected, the formulation of the compound
20 excipients, and its route of administration. In general terms, the compounds of this invention may be provided in an aqueous physiological buffer solution containing about 0.1 to 10% w/v compound for parenteral administration. Typical dose ranges are from about 10
25 ng/kg to about 1 g/kg of body weight per day; a preferred dose range is from about 0.1 μ g/kg to 100 mg/kg of body weight per day. Optimally, the morphogen dosage given in all cases is between 2-20 μ g of protein per kilogram weight of the patient per day.
30 No obvious OP-1 induced pathological lesions are induced when mature morphogen (e.g., OP-1, 20 μ g) is

administered daily to normal growing rats for
21 consecutive days. Moreover, 10 μ g systemic
injections of morphogen (e.g., OP-1) injected daily for
10 days into normal newborn mice does not produce any
5 gross abnormalities.

Since the ability of proteins and protein fragments
to penetrate the blood-brain barrier may be related to
their size, lipophilicity or their net ionic charge,
10 suitable modifications of the morphogens may be
formulated (e.g., by substituting
pentafluorophenylalanine for phenylalanine, or by
conjugation to a cationized protein such as albumin) to
increase their transportability across the barrier,
15 using standard methodologies known in the art. See,
for example, Kastin et al., Pharmac. Biochem. Behav.
(1979) 11:713-716; Rapoport et al., Science (1980)
207:84-86; Pardridge et al., (1987) Biochem. Biophys.
Res. Commun. 146:307-313; Riekkinen et al., (1987)
20 Peptides 8:261-265. The efficacy of these functional
analogues may be assessed for example, by evaluating the
ability of these compounds to induce redifferentiation
of transformed cells, or enhance survival of neurons at
risk of dying, as described in the Examples provided
25 herein.

In administering morphogens systemically in the
methods of the present invention, preferably a large
volume loading dose is used at the start of the
30 treatment. The treatment then is continued with a
maintenance dose. Further administration then can be
determined by monitoring at intervals the levels of the
morphogen in the blood.

Where injury to neurons of a neural pathway is induced deliberately as part of, for example, a surgical procedure, the morphogen preferably is provided just prior to, or concomitant with induction of the trauma. Preferably, the morphogen is administered prophylactically in a surgical setting. Optimally, the morphogen dosage given in all cases is between 2-20 μ g of protein per kilogram weight of the patient.

Alternatively, an effective amount of an agent capable of stimulating endogenous morphogen levels may be administered by any of the routes described above. For example, an agent capable of stimulating morphogen production and/or secretion from nerve tissue cells may be provided to a mammal, e.g., by direct administration of the morphogen to glial cells associated with the nerve tissue to be treated. A method for identifying and testing agents capable of modulating the levels of endogenous morphogens in a given tissue is described generally herein in Example 13, and in detail in copending USSN 752,859, filed August 30, 1991, the disclosure of which is incorporated herein by reference. Briefly, candidate compounds can be identified and tested by incubating the compound in vitro with a test tissue or cells thereof, for a time sufficient to allow the compound to affect the production, i.e., the expression and/or secretion, of a morphogen produced by the cells of that tissue. Here, suitable tissue or cultured cells of a tissue preferably would comprise neurons and/or glial cells. For example, suitable tissue for testing may include cultured cells isolated from the substantia nigra, adendema glial cells, and the like.

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A currently preferred detection means for
evaluating the level of the morphogen in culture upon
exposure to the candidate compound comprises an
immunoassay utilizing an antibody or other suitable
5 binding protein capable of reacting specifically with a
morphogen and being detected as part of a complex with
the morphogen. Immunoassays may be performed using
standard techniques known in the art and antibodies
raised against a morphogen and specific for that
10 morphogen. As described herein, morphogens may be
isolated from natural-sourced material or they may be
recombinantly produced. Agents capable of stimulating
endogenous morphogens then may be formulated into
pharmaceutical preparations and administered as
15 described herein.

Where the morphogen is to be provided to a site to
stimulate axon regeneration, the morphogen preferably
is provided to the site in association with a
20 biocompatible, preferably bioresorbable carrier
suitable for maintaining a protein at a site in vivo,
and through which a neural process may regenerate. A
currently preferred carrier also comprises sufficient
structure to assist direction of axonal growth.
25 Currently preferred carriers include structural
molecules such as collagen, hyaluronic acid or laminin,
and/or synthetic polymers or copolymers of, for
example, polylactic acid, polyglycolic acid or
polybutyric acid. Currently most preferred are
30 carriers comprising tissue extracellular matrix. These
may be obtained commercially. In addition, a brain
tissue-derived extracellular matrix may be prepared as
described in USSN 752,264, incorporated hereinabove by
reference, and/or by other means known in the art.

The currently preferred means for repairing breaks in neural pathways, particularly pathways of the peripheral nervous system, include providing the morphogen to the site as part of a device that includes a biocompatible membrane or casing of a dimension sufficient to span the break and having openings adapted to receive severed nerve ends. The morphogen is disposed within the casing, preferably dispersed throughout a suitable carrier, and is accessible to the severed nerve ends. Alternatively, the morphogen may be adsorbed onto the interior surface of the casing, or otherwise associated therewith. In addition, currently preferred casings have an impermeable exterior surface. The casing acts as a nerve guidance channel, aiding in directing axonal growth. In addition, the casing also protects the damaged nerve from immunologically-related agents which may assist in scar tissue formation. Suitable channel or casing materials include silicone or bioresorbable materials such as collagen, hyaluronic acid, laminin, polylactic acid, polyglycolic acid, polybutyric acid and the like. Additionally, although the nerve guidance channels described herein generally are tubular in shape, it should be evident to those skilled in the art that various alternative shapes may be employed. The lumen of the guidance channels may, for example, be oval or even square in cross section. Moreover the guidance channels may be constructed of two or more parts which may be clamped together to secure the nerve stumps. Nerve endings may be secured to the nerve guidance channels by means of sutures, biocompatible adhesives such as fibrin glue, or other means known in the medical art.

III. Examples

Example 1. Identification of Morphogen-Expressing Tissue

5

Determining the tissue distribution of morphogens may be used to identify different morphogens expressed in a given tissue, as well as to identify new, related morphogens. Tissue distribution also may be used to
10 identify useful morphogen-producing tissue for use in screening and identifying candidate morphogen-stimulating agents. The morphogens (or their mRNA transcripts) readily are identified in different tissues using standard methodologies and minor
15 modifications thereof in tissues where expression may be low. For example, protein distribution may be determined using standard Western blot analysis or immunofluorescent techniques, and antibodies specific to the morphogen or morphogens of interest. Similarly,
20 the distribution of morphogen transcripts may be determined using standard Northern hybridization protocols and transcript-specific probes.

Any probe capable of hybridizing specifically to a
25 transcript, and distinguishing the transcript of interest from other, related transcripts may be used. Because the morphogens described herein share such high sequence homology in their active, C-terminal domains, the tissue distribution of a specific morphogen
30 transcript may best be determined using a probe specific for the pro region of the immature protein and/or the N-terminal region of the mature protein. Another useful sequence is the 3' non-coding region flanking and immediately following the stop codon.
35 These portions of the sequence vary substantially among

the morphogens of this invention, and accordingly, are specific for each protein. For example, a particularly useful Vgr-1-specific probe sequence is the PvuII-SacI fragment, a 265 bp fragment encoding both a portion of the untranslated pro region and the N-terminus of the mature sequence (see Lyons et al. (1989) PNAS 86:4554-4558 for a description of the cDNA sequence). Similarly, particularly useful mOP-1-specific probe sequences are the BstXI-BglI fragment, a 0.68 Kb sequence that covers approximately two-thirds of the mOP-1 pro region; a StuI-StuI fragment, a 0.2 Kb sequence immediately upstream of the 7-cysteine domain; and the Earl-PstI fragment, an 0.3 Kb fragment containing a portion of the 3'untranslated sequence (See Seq. ID No. 18, where the pro region is defined essentially by residues 30-291.) Similar approaches may be used, for example, with hOP-1 (Seq. ID No. 16) or human or mouse OP-2 (Seq. ID Nos. 20 and 22.)

Using these morphogen-specific probes, which may be synthetically engineered or obtained from cloned sequences, morphogen transcripts can be identified in mammalian tissue, using standard methodologies well known to those having ordinary skill in the art. Briefly, total RNA is prepared from various adult murine tissues (e.g., liver, kidney, testis, heart, brain, thymus and stomach) by a standard methodology such as by the method of Chomczyaski et al. ((1987) Anal. Biochem 162:156-159) and described below. Poly (A)+ RNA is prepared by using oligo (dT)-cellulose chromatography (e.g., Type 7, from Pharmacia LKB Biotechnology, Inc.). Poly (A)+ RNA (generally 15 µg) from each tissue is fractionated on a 1% agarose/formaldehyde gel and transferred onto a Nytran membrane (Schleicher & Schuell). Following the

transfer, the membrane is baked at 80°C and the RNA is cross-linked under UV light (generally 30 seconds at 1 mW/cm²). Prior to hybridization, the appropriate probe is denatured by heating. The hybridization is carried out in a lucite cylinder rotating in a roller bottle apparatus at approximately 1 rev/min for approximately 15 hours at 37°C using a hybridization mix of 40% formamide, 5 x Denhardts, 5 x SSPE, and 0.1% SDS. Following hybridization, the non-specific counts are washed off the filters in 0.1 x SSPE, 0.1% SDS at 50°C.

Examples demonstrating the tissue distribution of various morphogens, including Vgr-1, OP-1, BMP2, BMP3, BMP4, BMP5, GDF-1, and OP-2 in developing and adult tissue are disclosed in co-pending USSN 752,764, and in Ozkaynak, et al., (1991) Biochem. Biophys. Res. Commun. 179:116-123, and Ozkaynak, et al. (1992) (JBC, in press), the disclosures of which are incorporated herein by reference. Using the general probing methodology described herein, northern blot hybridizations using probes specific for these morphogens to probe brain, spleen, lung, heart, liver and kidney tissue indicate that kidney-related tissue appears to be the primary expression source for OP-1, with brain, heart and lung tissues being secondary sources. Lung tissue appears to be the primary tissue expression source for Vgr-1, BMP5, BMP4 and BMP3. Lower levels of Vgr-1 also are seen in kidney and heart tissue, while the liver appears to be a secondary expression source for BMP5, and the spleen appears to be a secondary expression source for BMP4. GDF-1 appears to be expressed primarily in brain tissue. To date, OP-2 appears to be expressed primarily in early

embryonic tissue. Specifically, northern blots of murine embryos and 6-day post-natal animals shows abundant OP2 expression in 8-day embryos. Expression is reduced significantly in 17-day embryos and is not
5 detected in post-natal animals.

Example 2. Morphogen Localization in the Nervous System

10 Morphogens have been identified in developing and adult rat brain and spinal cord tissue, as determined both by northern blot hybridization of morphogen-specific probes to mRNA extracts from developing and adult nerve tissue (see Example 1, above) and by
15 immunolocalization studies. For example, northern blot analysis of developing rat tissue has identified significant OP-1 mRNA transcript expression in the CNS (USSN 752,764, and Ozkaynak et al. (1991) Biochem. Biophys. Res. Comm., 179:11623 and Ozkaynak, et al.
20 (1992) JBC, in press). GDF-1 mRNA appears to be expressed primarily in developing and adult nerve tissue, specifically in the brain, including the cerebellum and brain stem, spinal cord and peripheral nerves (Lee, S. (1991) PNAS 88: 4250-4254). BMP2B
25 (also referred in the art as BMP4) and Vgr-1 transcripts also have been reported to be expressed in nerve tissue (Jones et al. (1991) Development 111:531-542), although the nerve tissue does not appear to be the primary expression tissue for these genes
30 (Ozkaynak, et al., (1992) JBC in press). Specifically, CBMP2 transcripts are reported in the region of the diencephalon associated with pituitary development, and Vgr-1 transcripts are reported in the anteroposterior axis of the CNS, including in the roof plate of the
35 developing neural tube, as well as in the cells

immediately adjacent the floor plate of the developing neural tube. In older rats, Vgr-1 transcripts are reported in developing hippocampus tissue. In addition, the genes encoding OP-1 and BMP2 originally
5 were identified by probing human hippocampus cDNA libraries.

Immunolocalization studies, performed using standard methodologies known in the art and disclosed
10 in USSN 752,764, filed August 30, 1991, the disclosure of which is incorporated herein, localized OP-1 expression to particular areas of developing and adult rat brain and spinal cord tissue. Specifically, OP-1 protein expression was assessed in adult (2-3 months
15 old) and five or six-day old mouse embryonic nerve tissue, using standard morphogen-specific antisera, specifically, rabbit anti-OP1 antisera, made using standard antibody protocols known in the art and preferably purified on an OP-1 affinity column. The
20 antibody itself was labelled using standard fluorescent labelling techniques, or a labelled anti-rabbit IgG molecule was used to visualize bound OP-1 antibody.

As can be seen in FIG 1A and 1B, immunofluorescence
25 staining demonstrates the presence of OP-1 in adult rat central nervous system (CNS.) Similar and extensive staining is seen in both the brain (1A) and spinal cord (1B). OP-1 appears to be localized predominantly to the extracellular matrix of the grey matter (neuronal
30 cell bodies), distinctly present in all areas except the cell bodies themselves. In white matter, formed mainly of myelinated nerve fibers, staining appears to be confined to astrocytes (glial cells). A similar staining pattern also was seen in newborn rat (10 day
35 old) brain sections.

In addition, OP-1 has been specifically localized in the substantia nigra, which is composed primarily of striatal basal ganglia, a system of accessory motor neurons that function is association with the cerebral cortex and corticospinal system. Dysfunctions in this subpopulation or system of neurons are associated with a number of neuropathies, including Huntington's chorea and Parkinson's disease.

OP1 also has been localized at adenoma glial cells, known to secrete factors into the cerebrospinal fluid, and which occur around the intraventricular valve, choroid fissure, and central canal of the brain in both developing and adult rat.

Finally, morphogen inhibition in developing embryos inhibits nerve tissue development. Specifically, 9-day mouse embryo cells, cultured in vitro under standard culturing conditions, were incubated in the presence and absence of an OP-1-specific monoclonal antibody prepared using recombinantly produced, purified mature OP-1 and the immunogen. The antibody was prepared using standard antibody production means well known in the art and as described generally in Example 13. After two days, the effect of the antibody on the developing embryo was evaluated by histology. As determined by histological examination, the OP-1-specific antibody specifically inhibits eye lobe formation in the developing embryo. In particular, the diencephalon outgrowth does not develop. In addition, the heart is malformed and enlarged. Moreover, in separate immunolocalization studies on embryo sections with labelled OP-1 specific antibody, the OP-1-specific antibody localizes to neural epithelia.

The endogenous morphogens which act on neuronal cells may be expressed and secreted by nerve tissue cells, e.g., by neurons and/or glial cells associated with the neurons, and/or they may be transported to the neurons by the cerebrospinal fluid and/or bloodstream. Recently, OP-1 has been identified in the human blood (See Example 9, below). In addition, transplanted Schwann cells recently have been shown to stimulate nerve fiber formation in rat spinal cord, including inducing vascularization and myelin sheath formation around at least some of the new neuronal processes (Bunge (1991) Exp. Neurology 114:254-257.) The regenerative property of these cells may be mediated by the secretion of a morphogen by the Schwann cells.

Example 3. Morphogen Enhancement of Neuronal Cell Survival

The morphogens described herein enhance cell survival, particularly of neuronal cells at risk of dying. For example, fully differentiated neurons are non-mitotic and die in vitro when cultured under standard mammalian cell culture conditions, using a chemically defined or low serum medium known in the art, (see, for example, Charness (1986) J. Biol. Chem. 26:3164-3169 and Freese et al. (1990) Brain Res. 521:254-264.) However, if a primary culture of non-mitotic neuronal cells is treated with a morphogen, the survival of these cells is enhanced significantly. For example, a primary culture of striatal basal ganglia isolated from the substantia nigra of adult rat brain was prepared using standard procedures, e.g., by dissociation by trituration with pasteur pipette of substantia nigra tissue, using standard tissue culturing protocols, and grown in a low serum medium, e.g.,

containing 50% DMEM (Dulbecco's modified Eagle's medium), 50% F-12 medium, heat inactivated horse serum supplemented with penicillin/streptomycin and 4 g/l glucose. Under standard culture conditions, these
5 cells are undergoing significant cell death by three weeks when cultured in a serum-free medium. Cell death is evidenced morphologically by the inability of cells to remain adherent and by changes in their ultrastructural characteristics, e.g., by chromatin
10 clumping and organelle disintegration.

In this example, the cultured basal ganglia were were treated with chemically defined medium conditioned with 0.1-100 ng/ml OP-1. Fresh, morphogen-conditioned
15 medium was provided to the cells every 3-4 days. Cell survival was enhanced significantly and was dose dependent upon the level of OP-1 added: cell death decreased significantly as the concentration of OP-1 was increased in cell cultures. Specifically, cells
20 remained adherent and continued to maintain the morphology of viable differentiated neurons. In the absence of morphogen treatment, the majority of the cultured cells dissociated and underwent cell necrosis.

Dysfunctions in the basal ganglia of the substantia nigra are associated with Huntington's chorea and parkinsonism in vivo. The ability of the morphogens defined herein to enhance neuron survival indicates that these morphogens will be useful as part of a
30 therapy to enhance survival of neuronal cells at risk of dying in vivo due, for example, to a neuropathy or chemical or mechanical trauma. It is particularly anticipated that these morphogens will provide a useful therapeutic agent to treat neuropathies which affect
35 the striatal basal ganglia, including Huntington's

chorea and Parkinson's disease. For clinical applications, the morphogen may be administered or, alternatively, a morphogen-stimulating agent may be administered.

5

Example 4. Morphogen-Induced Redifferentiation of Transformed Cells

10 The morphogens described herein also induce redifferentiation of transformed cells to a morphology characteristic of untransformed cells. In particular, the morphogens are capable of inducing redifferentiation of transformed cells of neuronal
15 origin to a morphology characteristic of untransformed neurons. The example provided below details morphogen induced redifferentiation of a transformed human cell line of neuronal origin, NG105-115. Morphogen-induced redifferentiation of transformed cells also has been
20 shown in mouse neuroblastoma cells (N1E-115) and in human embryo carcinoma cells (see copending USSN 752,764, incorporated herein by reference.)

NG108-15 is a transformed hybrid cell line produced
25 by fusing neuroblastoma x glioma cells (obtained from American Type Tissue Culture, Rockville, MD), and exhibiting a morphology characteristic of transformed embryonic neurons, e.g., having a fibroblastic morphology. Specifically, the cells have polygonal
30 cell bodies, short, spike-like processes and make few contacts with neighboring cells (see FIG. 1A). Incubation of NG108-15 cells, cultured in a chemically defined, serum-free medium, with 0.1 to 300 ng/ml of OP-1 for four hours induces an orderly, dose-dependent
35 change in cell morphology.

In the experiment NG108-15 cells were subcultured on poly-L-lysine coated 6-well plates. Each well contained 40-50,000 cells in 2.5 ml of chemically defined medium. On the third day 2.5 μ l of OP-1 in 60% ethanol containing 0.025% trifluoroacetic was added to each well. OP-1 concentrations of 0-300 ng/ml were tested. Typically, the media was changed daily with new aliquots of OP-1, although morphogenesis can be induced by a single four hour incubation with OP-1. In addition, OP-1 concentrations of 10 ng/ml were sufficient to induce redifferentiation. After one day, hOP-1-treated cells undergo a significant change in their cellular ultrastructure, including rounding of the soma, increase in phase brightness and extension of the short neurite processes. After two days, cells treated with OP-1 begin to form epithelioid sheets, which provide the basis for the growth of multilayered aggregates at three day's post-treatment. By four days, the great majority of OP-1-treated cells are associated in tightly-packed, multilayered aggregates (Fig. 1B). Fig. 2 plots the mean number of multilayered aggregates or cell clumps identified in twenty randomly selected fields from six independent experiments, as a function of morphogen concentration. Forty ng/ml of OP-1 is sufficient for half maximum induction of cell aggregation.

The morphogen-induced redifferentiation occurred without any associated changes in DNA synthesis, cell division, or cell viability, making it unlikely that the morphologic changes were secondary to cell differentiation or a toxic effect of hOP-1. Moreover, the OP-1-induced morphogenesis does not inhibit cell division, as determined by ^3H -thymidine uptake, unlike other molecules which have been shown to stimulate

differentiation of transformed cells, such as butyrate, DMSO, retanoic acid or Forskolin. The data indicate that OP-1 can maintain cell stability and viability after inducing redifferentiation. In addition, the effects are morphogen specific, and redifferentiation is not induced when NG108-15 cells are incubated with 0.1-40 ng/ml TGF- β .

The experiments also have been performed with highly purified soluble morphogen (e.g., mature OP1 associated with its pro domain) which also specifically induced redifferentiation of NG108-15 cells.

The morphogens described herein accordingly provide useful therapeutic agents for the treatment of neoplasias and neoplastic lesions of the nervous system, particularly in the treatment of neuroblastomas, including retinoblastomas, and gliomas. The morphogens themselves may be administered or, alternatively, a morphogen-stimulating agent may be administered.

Example 5. Nerve Tissue Protection from Chemical Trauma

The ability of the morphogens described herein to enhance survival of neuronal cells and to induce cell aggregation and cell-cell adhesion in redifferentiated cells, indicates that the morphogens will be useful as therapeutic agents to maintain neural pathways by protecting the cells defining the pathway from the damage caused by chemical trauma. In particular, the morphogens can protect neurons, including developing neurons, from the effects of toxins known to inhibit

5 The toxic effects of ethanol on developing neurons induces the neurological damage manifested in fetal alcohol syndrome. The morphogens also may protect neurons from the cytotoxic effects associated with excitatory amino acids such as glutamate.

For example, ethanol inhibits the cell-cell adhesion effects induced in morphogen-treated NG108-15 cells when provided to these cells at a concentration of 25-50 mM. Half maximal inhibition can be achieved with 5-10 mM ethanol, the concentration of blood alcohol in an adult following ingestion of a single alcoholic beverage. Ethanol likely interferes with the homophilic binding of CAMs between cells, rather than their induction, as morphogen-induced N-CAM levels are unaffected by ethanol. Moreover, the inhibitory effect is inversely proportional to morphogen concentration. Accordingly, it is envisioned that administration of a morphogen or morphogen-stimulating agent to neurons, particularly developing neurons, at risk of damage from exposure to toxins such as ethanol, may protect these cells from nerve tissue damage by overcoming the toxin's inhibitory effects. The morphogens described herein also are useful in therapies to treat damaged neural pathways resulting from a neuropathy induced by exposure to these toxins.

Example 6. Morphogen-Induced CAM Expression

5 The morphogens described herein induce CAM
expression, particularly N-CAM expression, as part of
their induction of morphogenesis. CAMs are
morphoregulatory molecules identified in all tissues as
an essential step in tissue development. N-CAMs, which
comprise at least 3 isoforms (N-CAM-180, N-CAM-140 and
N-CAM-120, where "180", "140" and "120" indicate the
10 apparent molecular weights of the isoforms as measured
by polyacrylamide gel electrophoresis) are expressed at
least transiently in developing tissues, and
permanently in nerve tissue. Both the N-CAM-180 and N-
CAM-140 isoforms are expressed in both developing and
15 adult tissue. The N-CAM-120 isoform is found only in
adult tissue. Another neural CAM is L1.

20 N-CAMs are implicated in appropriate neural
development, including appropriate nuerulation,
neuronal migration, fasciculation, and synaptogenesis.
Inhibition of N-CAM production, as by complexing the
molecule with an N-CAM-specific antibody, inhibits
retina organization, including retinal axon migration,
and axon regeneration in the peripheral nervous system,
25 as well as axon synapsis with target muscle cells. In
addition, significant evidence indicates that physical
or chemical trauma to neurons, oncogenic transformation
and some genetic neurological disorders are accompanied
by changes in CAM expression, which alter the adhesive
30 or migratory behavior of these cells. Specifically,
increased N-CAM levels are reported in Huntington's
disease striatum (e.g., striatal basal ganglia), and
decreased adhesion is noted in Alzheimer's disease.

The morphogens described herein can stimulate CAM production, particularly L1 and N-CAM production, including all three isoforms of the N-CAM molecule. For example, N-CAM expression is stimulated

5 significantly in morphogen-treated NG108-15 cells. Untreated NG108-15 cells exhibit a fibroblastic, or minimally differentiated morphology and express only the 180 and 140 isoforms of N-CAM normally associated with a developing cell. Following morphogen treatment

10 these cells exhibit a morphology characteristic of adult neurons and express enhanced levels of all three N-CAM isoforms. Using a similar protocol as described in the example below, morphogen treatment of NG108-15 cells also induced L1 expression.

15

In this example NG108-15 cells were cultured for 4 days in the presence of increasing concentrations of OP-1 and standard Western blots performed on whole cells extracts. N-CAM isoforms were detected with an

20 antibody which crossreacts with all three isoforms, mAb H28.123, obtained from Sigma Chemical Co., St. Louis, the different isoforms being distinguishable by their different mobilities on an electrophoresis gel. Control NG108-15 cells (untreated) express both

25 the 140 kDa and the 180 kDa isoforms, but not the 120 kDa, as determined by western blot analyses using up to 100 μ g of protein. Treatment of NG108-15 cells with OP-1 resulted in a dose-dependent increase in the expression of the 180 kDa and 140 kDa isoforms, as well

30 as the induction of the 120 kDa isoform. See Fig. 2A and 2B. Fig. 2B is a Western blot of OP1-treated NG108-15 cell extracts, probed with mAb H28.123, showing the induction of all three isoforms. Fig. 2A is a dose response curve of N-CAM-180 and N-CAM-140

35 induction as a function of morphogen concentration. N-

CAM-120 is not shown in the graph as it could not be quantitated in control cells. However, as is clearly evident from the Western blot in Fig. 2A, N-CAM-120 is induced in response to morphogen treatment. The
5 differential induction of N-CAM 180 and 140 isoforms seen may be because constitutive expression of the 140 isoform is close to maximum.

The increase in N-CAM expression corresponded in a
10 dose-dependent manner with the morphogen induction of multicellular aggregates. Compare Fig. 2A and Fig 3. Fig. 3 graphs the mean number of multilayered aggregates (clumps) counted per 20 randomly selected fields in 6 independent experiments, versus the
15 concentration of morphogen. The induction of the 120 isoform also indicates that morphogen-induced redifferentiation of transformed cells stimulates not only redifferentiation of these cells from a transformed phenotype, but also differentiation to a
20 phenotype corresponding to a developed cell. Standard immunolocalization studies performed with the mAb H28.123 on morphogen-treated cells show N-CAM cluster formation associated with the periphery and processes of treated cells and no reactivity with untreated
25 cells. Moreover, morphogen treatment does not appear to inhibit cell division as determined by cell counting or ³H-thymidine uptake. Finally, known chemical differentiating agents, such as Forskolin and dimethylsulfoxide do not induce N-CAM production.

30

In addition, the cell aggregation effects of OP-1 on NG108-15 cells can be inhibited with anti-N-CAM antibodies or antisense N-CAM oligonucleotides. Antisense oligonucleotides can be made synthetically on
35 a nucleotide synthesizer, using standard means known in

the art. Preferably, phosphorothioate oligonucleotides ("S-oligos") are prepared, to enhance transport of the nucleotides across cell membranes. Concentrations of both N-CAM antibodies and N-CAM antisense

5 oligonucleotides sufficient to inhibit N-CAM induction also inhibited formation of multilayered cell aggregates. Specifically, incubation of morphogen-treated NG108-115 cells with 0.3-3 μ M N-CAM antisense S-oligos, 5-500 μ M unmodified N-CAM antisense oligos,
10 or 10 μ g/ml mAb H28.123 significantly inhibits cell aggregation. It is likely that morphogen treatment also stimulates other CAMs, as inhibition is not complete.

15 The experiments also have been performed with soluble morphogen (e.g., mature OP-1 associated with its pro domain) which also specifically induced CAM expression.

20 The morphogens described herein are useful as therapeutic agents to treat neurological disorders associated with altered CAM levels, particularly N-CAM levels, such as Huntington's chorea and Alzheimers' disease, and the like. In clinical applications, the
25 morphogens themselves may be administered or, alternatively, a morphogen-stimulating agent may be administered.

The efficacy of the morphogens described herein to
30 affect N-CAM expression may be assessed in vitro using a suitable cell line and the methods described herein. In addition to a transformed cell line, N-CAM expression can be assayed in a primary cell culture of neural or glial cells, following the procedures
35 described herein. The efficacy of morphogen treatment

on N-CAM expression in vivo may be evaluated by tissue biopsy as described in Example 9, below, and detecting N-CAM molecules with an N-CAM-specific antibody, such as mAb H28.123, or using the animal model described in
5 Example 11.

Alternatively, the level of N-CAM proteins or protein fragments present in cerebrospinal fluid or serum also may be detected to evaluate the effect of
10 morphogen treatment. N-CAM molecules are known to slough off cell surfaces and have been detected in both serum and cerebrospinal fluid. In addition, altered levels of the soluble form of N-CAM are associated with normal pressure hydrocephalus and type II
15 schizophrenia. N-CAM fluid levels may be detected following the procedure described in Example 9 and using an N-CAM specific antibody, such as mAb H28.123.

20 Example 7. Morphogen-Induced Nerve Gap Repair (PNS)

The morphogens described herein also stimulate peripheral nervous system axonal growth over extended distances allowing repair and regeneration of damaged
25 neural pathways. While neurons of the peripheral nervous system can sprout new processes following injury, without guidance these sproutings typically fail to connect appropriately and die. Where the break is extensive, e.g., greater than 5 or 10 mm,
30 regeneration is poor or nonexistent.

In this example morphogen stimulation of nerve regeneration was assessed using the rat sciatic nerve model. The rat sciatic nerve can regenerate
35 spontaneously across a 5 mm gap, and occasionally

across a 10 mm gap, provided that the severed ends are inserted in a saline-filled nerve guidance channel. In this experiment, nerve regeneration across a 12mm gap was tested.

5

Adult female Sprague-Dawley rats (Charles River Laboratories, Inc.) weighing 230-250 g were anesthetized with intraperitoneal injections of sodium pentobarbital 35 mg/kg body weight). A skin incision
10 was made parallel and just posterior to the femur. The avascular intermuscular plane between vastus lateralis and hamstring muscles were entered and followed to the loose fibroareolar tissue surrounding the sciatic nerve. The loose tissue was divided longitudinally
15 thereby freeing the sciatic nerve over its full extent without devascularizing any portion. Under a surgical microscope the sciatic nerves were transected with microscissors at mid-thigh and grafted with an OP-1 gel graft that separated the nerve stumps by 12 mm. The
20 graft region was encased in a silicone tube 20 mm in length with a 1.5 mm inner diameter, the interior of which was filled a morphogen solution. Specifically, The central 12 mm of the tube consisted of an OP-1 gel prepared by mixing 1 to 5 μ g of substantially pure CHO-
25 produced recombinant OP-1 with approximately 100 μ l of MATRIGELTM (from Collaborative Research, Inc., Bedford, MA), an extracellular matrix extract derived from mouse sarcoma tissue, and containing solubilized tissue basement membrane, including laminin, type IV collagen,
30 heparin sulfate, proteoglycan and entactin, in phosphate-buffered saline. The OP-1-filled tube was implanted directly into the defect site, allowing 4 mm

on each end to insert the nerve stumps. Each stump was abutted against the OP-1 gel and was secured in the silicone tube by three stitches of commercially available surgical 10-0 nylon through the epineurium,
5 the fascicle protective sheath.

In addition to OP-1 gel grafts, empty silicone tubes, silicone tubes filled with gel only and "reverse" autografts, wherein 12 mm transected segments
10 of the animal's sciatic nerve were rotated 180° prior to suturing, were grafted as controls. All experiments were performed in quadruplicate. All wounds were closed by wound clips that were removed after 10 days. All rats were grafted on both legs. At 3 weeks the
15 animals were sacrificed, and the grafted segments removed and frozen on dry ice immediately. Frozen sections then were cut throughout the graft site, and examined for axonal regeneration by immunofluorescent staining using anti-neurofilament antibodies labeled
20 with flurocein (obtained from Sigma Chemical Co., St. Louis).

Regeneration of the sciatic nerve occurred across the entire 12 mm distance in all graft sites wherein
25 the gap was filled with the OP-1 gel. By contrast, empty silicone tubes and reverse autografts did not show nerve regeneration, and only one graft site containing the gel alone showed axon regeneration.

Example 8. Morphogen-Induced Nerve Gap Repair (CNS)

Following axonal damage in vivo the CNS neurons are
unable to resprout processes. Accordingly, trauma to
5 CNS nerve tissue, including the spinal cord, optic
nerve and retina, severely damages or destroys the
neural pathways defined by these cells. Peripheral
nerve grafts have been used in an effort to bypass CNS
axonal damage. Successful autologous graft repair to
10 date apparently requires that the graft site occur near
the CNS neuronal cell body, and a primary result of CNS
axotomy is neuronal cell death. The efficacy of
morphogens described herein on CNS nerve repair, may be
evaluated using a rat crushed optic nerve model such as
15 the one described by Bignami et al., (1979) Exp. Eye
Res. 28: 63-69, the disclosure of which is incorporated
herein by reference. Briefly, and as described
therein, laboratory rats (e.g., from Charles River
Laboratories, Wilmington, MA) are anesthetized using
20 standard surgical procedures, and the optic nerve
crushed by pulling the eye gently out of the orbit,
inserting a watchmaker forceps behind the eyeball and
squeezing the optic nerve with the forceps for
15 seconds, followed by a 30 second interval and second
25 15 second squeeze. Rats are sacrificed at different
time intervals, e.g., at 48 hours, and at 3, 4, 11, 15
and 18 days after operation. The effect of morphogen
on optic nerve repair can be assessed by performing the
experiment in duplicate and providing morphogen or PBS
30 (e.g., 25 μ l solution, and 25 μ g morphogen) to the
optic nerve, e.g., just prior to the operation,
concomitant with the operation, or at specific times
after the operation.

In the absence of therapy, the surgery induces glial scarring of the crushed nerve, as determined by immunofluorescence staining for glial fibrillary acidic protein (GFA), a marker protein for glial scarring, and
5 by histology. Indirect immunofluorescence on air-dried cryostat sections as described in Bignami et al. (1974) J. Comp. Neur. 153: 27-38, using commercially available antibodies to GFA (e.g., Sigma Chemical Co., St. Louis). Reduced levels of GFA are anticipated in
10 animals treated with the morphogen, evidencing the ability of morphogens to inhibit glial scar formation and to stimulate optic nerve regeneration.

15 Example 9. Nerve Tissue Diagnostics

Morphogen localization in nerve tissue can be used as part of a method for diagnosing a neurological disorder or neuropathy. The method may be particularly
20 advantageous for diagnosing neuropathies of brain tissue. Specifically, a biopsy of brain tissue is performed on a patient at risk, using standard procedures known in the medical art. Morphogen expression associated with the biopsied tissue then is
25 assessed using standard methodologies, as by immunolocalization, using standard immunofluorescence techniques in concert with morphogen-specific antisera or monoclonal antibodies. Specifically, the biopsied tissue is thin sectioned using standard methodologies
30 known in the art, and fluorescently labelled (or otherwise detectable) antibodies incubated with the tissue under conditions sufficient to allow specific antigen-antibody complex formation. The presence and quantity of complex formed then is detected and
35 compared with a predetermined standard or reference

value. Detection of altered levels of morphogen present in the tissue then may be used as an indicator of tissue dysfunction. Alternatively, fluctuation in morphogen levels may be assessed by monitoring
5 morphogen transcription levels, either by standard northern blot analysis or in situ hybridization, using a labelled probe capable of hybridizing specifically to morphogen RNA and standard RNA hybridization protocols well described in the art.

10

Fluctuations in morphogen levels present in the cerebrospinal fluid or bloodstream also may be used to evaluate nerve tissue viability. For example, morphogens are detected associated with adendema cells
15 which are known to secrete factors into the cerebrospinal fluid, and are localized generally associated with glial cells, and in the extracellular matrix, but not with neuronal cell bodies. Accordingly, the cerebrospinal fluid may be a natural
20 means of morphogen transport. Alternatively, morphogens may be released from dying cells into cerebrospinal fluid. In addition, OP-1 recently has been identified in human blood, which also may be a means of morphogen transport, and/or a repository for
25 the contents of dying cells.

Spinal fluid may be obtained from an individual by a standard lumbar puncture, using standard methodologies known in the medical art. Similarly,
30 serum samples may be obtained by standard venipuncture and serum prepared by centrifugation at 3,000 RPM for ten minutes. The presence of morphogen in the serum or cerebral spinal fluid then may be assessed by standard Western blot (immunoblot), ELISA or RIA procedures.
35 Briefly, for example, with the ELISA, samples may be

diluted in an appropriate buffer, such as phosphate-buffered saline, and 50 μ l aliquots allowed to absorb to flat bottomed wells in microtitre plates pre-coated with morphogen-specific antibody, and allowed to
5 incubate for 18 hours at 4°C. Plates then may be washed with a standard buffer and incubated with 50 μ l aliquots of a second morphogen-specific antibody conjugated with a detecting agent, e.g., biotin, in an appropriate buffer, for 90 minutes at room temperature.
10 Morphogen-antibody complexes then may be detected using standard procedures.

Alternatively, a morphogen-specific affinity column may be created using, for example, morphogen-specific
15 antibodies adsorbed to a column matrix, and passing the fluid sample through the matrix to selectively extract the morphogen of interest. The morphogen then is eluted. A suitable elution buffer may be determined empirically by determining appropriate binding and
20 elution conditions first with a control (e.g., purified, recombinantly-produced morphogen.) Fractions then are tested for the presence of the morphogen by standard immunoblot, and confirmed by N-terminal sequencing. Morphogen concentrations in serum or other
25 fluid samples then may be determined using standard protein quantification techniques, including by spectrophotometric absorbance or by quantitation by ELISA or RIA antibody assays. Using this procedure, OP-1 has been identified in serum.

30

OP-1 was detected in human serum using the following assay. A monoclonal antibody raised against mammalian, recombinantly produced OP-1 using standard immunology techniques well described in the art and
35 described generally in Example 13, was immobilized by

passing the antibody over an activated agarose gel
(e.g., Affi-GelTM, from Bio-Rad Laboratories, Richmond,
CA, prepared following manufacturer's instructions),
and used to purify OP-1 from serum. Human serum then
5 was passed over the column and eluted with 3M
K-thiocyanate. K-thiocyanate fractions then were
dialyzed in 6M urea, 20mM PO₄, pH 7.0, applied to a C8
HPLC column, and eluted with a 20 minute, 25-50%
acetonitrile/0.1% TFA gradient. Mature, recombinantly
10 produced OP-1 homodimers elute between 20-22 minutes.
Fractions then were collected and tested for the
presence of OP-1 by standard immunoblot. Fig. 4 is an
immunoblot showing OP-1 in human sera under reducing
and oxidized conditions. In the figure, lanes 1 and 4
15 are OP-1 standards, run under oxidized (lane 1) and
reduced (lane 4) conditions. Lane 5 shows molecular
weight markers at 17, 27 and 39 kDa. Lanes 2 and 3 are
human sera OP-1, run under oxidized (lane 2) and
reduced (lane 3) conditions.

20

Morphogens may be used in diagnostic applications
by comparing the quantity of morphogen present in a
body fluid sample with a predetermined reference value,
with fluctuations in fluid morphogen levels indicating
25 a change in the status of nerve tissue. Alternatively,
fluctuations in the level of endogenous morphogen
antibodies may be detected by this method, most likely
in serum, using an antibody or other binding protein
capable of interacting specifically with the endogenous
30 morphogen antibody. Detected fluctuations in the
levels of the endogenous antibody may be used as
indicators of a change in tissue status.

Example 10. Alleviation of Immune Response-Mediated
Nerve Tissue Damage

269360" 95426680

The morphogens described herein may be used to
5 alleviate immunologically-related damage to nerve
tissue. Details of this damage and the use of
morphogens to alleviate this injury are disclosed in
copending USSN 753,059, filed August 30, 1991, the
disclosure of which is incorporated herein. A primary
10 source of such damage to nerve tissue follows hypoxia
or ischemia-reperfusion of a blood supply to a neural
pathway, such as may result from an embolic stroke, or
may be induced during a surgical procedure. As
described in USSN 753,059, morphogens have been shown
15 to alleviate damage to myocardial tissue following
ischemia-reperfusion of the blood supply to the tissue.
The effect of morphogens on alleviating
immunologically-related damage to nerve tissue may be
assessed using methodologies and models known to those
20 skilled in the art and described below.

For example, the rabbit embolic stroke model
provides a useful method for assessing the effect of
morphogens on tissue injury following cerebral
25 ischemia-reperfusion. The protocol disclosed below is
essentially that of Phillips et al. (1989) Annals of
Neurology 25:281-285, the disclosure of which is herein
incorporated by reference. Briefly, white New England
rabbits (2-3kg) are anesthetized and placed on a
30 respirator. The intracranial circulation then is
selectively catheterized by the Seldinger technique.
Baseline cerebral angiography then is performed,
employing a digital substration unit. The distal
internal carotid artery or its branches then is
35 selectively embolized with 0.035 ml of 18-hour-aged

autologous thrombus. Arterial occlusion is documented by repeat angiography immediately after embolization. After a time sufficient to induce cerebral infarcts (15 minutes or 90 minutes), reperfusion is induced by
5 administering a bolus of a reperfusion agent such as the TPA analogue FB-FB-CF (e.g., 0.8 mg/kg over 2 minutes).

The effect of morphogen on cerebral infarcts can be
10 assessed by administering varying concentrations of morphogens, e.g., OP-1, at different times following embolization and/or reperfusion. The rabbits are sacrificed 3-14 days post embolization and their brains prepared for neuropathological examination by fixing by
15 immersion in 10% neutral buffered formalin for at least 2 weeks. The brains then are sectioned in a coronal plane at 2-3 mm intervals, numbered and submitted for standard histological processing in paraffin, and the degree of nerve tissue necrosis
20 determined visually. Morphogen-treated animals are anticipated to reduce or significantly inhibit nerve tissue necrosis following cerebral ischemia-reperfusion in the test animals as determined by histology comparison with nontreated animals.

25

Example 11. Animal Model for Assessing Morphogen
Efficacy In Vivo

The in vivo activities of the morphogens described
30 herein also are assessed readily in an animal model as described herein. A suitable animal, preferably exhibiting nerve tissue damage, for example, genetically or environmentally induced, is injected intracerebrally with an effective amount of a morphogen
35 in a suitable therapeutic formulation, such as

phosphate-buffered saline, pH 7. The morphogen preferably is injected within the area of the affected neurons. The affected tissue is excised at a subsequent time point and the tissue evaluated morphologically and/or by evaluation of an appropriate biochemical marker (e.g., by morphogen or N-CAM localization; or by measuring the dose-dependent effect on a biochemical marker for CNS neurotrophic activity or for CNS tissue damage, using for example, glial fibrillary acidic protein as the marker. The dosage and incubation time will vary with the animal to be tested. Suitable dosage ranges for different species may be determined by comparison with established animal models. Presented below is an exemplary protocol for a rat brain stab model.

Briefly, male Long Evans rats, obtained from standard commercial sources, are anesthetized and the head area prepared for surgery. The calvariae is exposed using standard surgical procedures and a hole drilled toward the center of each lobe using a 0.035K wire, just piercing the calvariae. 25 μ l solutions containing either morphogen (e.g., OP-1, 25 μ g) or PBS then is provided to each of the holes by Hamilton syringe. Solutions are delivered to a depth approximately 3 mm below the surface, into the underlying cortex, corpus callosum and hippocampus. The skin then is sutured and the animal allowed to recover.

30

Three days post surgery, rats are sacrificed by decapitation and their brains processed for sectioning. Scar tissue formation is evaluated by immunofluorescence staining for glial fibrillary acidic protein, a marker protein for glial scarring, to qualitatively determine

35

the degree of scar formation. Glial fibrillary acidic protein antibodies are available commercially, e.g., from Sigma Chemical Co., St. Louis, MO. Sections also are probed with anti-OP-1 antibodies to determine the presence of OP-1. Reduced levels of glial fibrillary acidic protein are anticipated in the tissue sections of animals treated with the morphogen, evidencing the ability of morphogens to inhibit glial scar formation and stimulate nerve regeneration.

10

Example 12. In Vitro Model for Evaluating Morphogen Species Transport Across the Blood-Brain Barrier.

15

Described below is an in vitro method for evaluating the facility with which selected morphogen species likely will pass across the blood-brain barrier. A detailed description of the model and protocol are provided by Audus et al. (1987) Ann. N.Y.

20

Acad. Sci. 507:9-18, the disclosure of which is incorporated herein by reference.

Briefly, microvessel endothelial cells are isolated from the cerebral gray matter of fresh bovine brains. Brains are obtained from a local slaughter house and transported to the laboratory in ice cold minimum essential medium (MEM) with antibiotics. Under sterile conditions the large surface blood vessels and meninges are removed using standard dissection procedures. The cortical gray matter is removed by aspiration, then minced into cubes of about 1mm. The minced gray matter then is incubated with 0.5% dispase (BMB, Indianapolis, IN) for 3 hours at 37° C in a shaking water bath. Following the 3 hour digestion, the mixture is concentrated by centrifugation (1000 x g for 10 min.),

then resuspended in 13% dextran and centrifuged for 10 min. at 5800 x g. Supernatant fat, cell debris and myelin are discarded and the crude microvessel pellet resuspended in 1 mg/ml collagenase/dispase and
5 incubated in a shaking water bath for 5 hours at 37° C. After the 5-hour digestion, the microvessel suspension is applied to a pre-established 50% Percoll gradient and centrifuged for 10 min at 1000 x g. The band
10 containing purified endothelial cells (second band from the top of the gradient) is removed and washed two times with culture medium (e.g., 50% MEM/50% F-12 nutrient mix). The cells are frozen (-80° C.) in medium containing 20% DMSO and 10% horse serum for later use.

15 After isolation, approximately 5×10^5 cells/cm² are plated on culture dishes or 5-12 μ pore size polycarbonate filters that are coated with rat collagen and fibronectin. 10-12 days after seeding the cells,
20 cell monolayers are inspected for confluency by microscopy.

Characterization of the morphological, histochemical and biochemical properties of these cells
25 has shown that these cells possess many of the salient features of the blood-brain barrier. These features include: tight intercellular junctions, lack of membrane fenestrations, low levels of pinocytotic activity, and the presence of gamma-glutamyl
30 transpeptidase, alkaline phosphatase, and Factor VIII antigen activities.

The cultured cells can be used in a wide variety of experiments where a model for polarized binding or
35 transport is required. By plating the cells in

multi-well plates, receptor and non-receptor binding of both large and small molecules can be conducted. In order to conduct transendothelial cell flux measurements, the cells are grown on porous polycarbonate membrane filters (e.g., from Nucleopore, Pleasanton, CA). Large pore size filters (5-12 μ) are used to avoid the possibility of the filter becoming the rate-limiting barrier to molecular flux. The use of these large-pore filters does not permit cell growth under the filter and allows visual inspection of the cell monolayer.

Once the cells reach confluency, they are placed in a side-by-side diffusion cell apparatus (e.g., from Crown Glass, Sommerville, NJ). For flux measurements, the donor chamber of the diffusion cell is pulsed with a test substance, then at various times following the pulse, an aliquot is removed from the receiver chamber for analysis. Radioactive or fluorescently-labelled substances permit reliable quantitation of molecular flux. Monolayer integrity is simultaneously measured by the addition of a non-transportable test substance such as sucrose or inulin and replicates of at least 4 determinations are measured in order to ensure statistical significance.

Example 13. Screening Assay for Candidate Compounds which Alter Endogenous Morphogen Levels

Candidate compound(s) which may be administered to affect the level of a given morphogen may be found using the following screening assay, in which the level of morphogen production by a cell type which produces measurable levels of the morphogen is determined with and without incubating the cell in culture with the

compound, in order to assess the effects of the
compound on the cell. This can be accomplished by
detection of the morphogen either at the protein or RNA
level. A more detailed description also may be found
5 in USSN 752,861, incorporated hereinabove by reference.

13.1 Growth of Cells in Culture

Cell cultures of kidney, adrenals, urinary bladder,
10 brain, or other organs, may be prepared as described
widely in the literature. For example, kidneys may be
explanted from neonatal or new born or young or adult
rodents (mouse or rat) and used in organ culture as
whole or sliced (1-4 mm) tissues. Primary tissue
15 cultures and established cell lines, also derived from
kidney, adrenals, urinary, bladder, brain, mammary, or
other tissues may be established in multiwell plates (6
well or 24 well) according to conventional cell culture
techniques, and are cultured in the absence or presence
20 of serum for a period of time (1-7 days). Cells may be
cultured, for example, in Dulbecco's Modified Eagle
medium (Gibco, Long Island, NY) containing serum (e.g.,
fetal calf serum at 1%-10%, Gibco) or in serum-deprived
medium, as desired, or in defined medium (e.g.,
25 containing insulin, transferrin, glucose, albumin, or
other growth factors).

Samples for testing the level of morphogen
production includes culture supernatants or cell
30 lysates, collected periodically and evaluated for OP-1
production by immunoblot analysis (Sambrook et al.,
eds., 1989, Molecular Cloning, Cold Spring Harbor
Press, Cold Spring Harbor, NY), or a portion of the
cell culture itself, collected periodically and used to
35 prepare polyA+ RNA for RNA analysis. To monitor de

novo OP-1 synthesis, some cultures are labeled according to conventional procedures with an ^{35}S -methionine/ ^{35}S -cysteine mixture for 6-24 hours and then evaluated to OP-1 synthesis by conventional immunoprecipitation methods.

13.2 Determination of Level of Morphogenic Protein

In order to quantitate the production of a morphogenic protein by a cell type, an immunoassay may be performed to detect the morphogen using a polyclonal or monoclonal antibody specific for that protein. For example, OP-1 may be detected using a polyclonal antibody specific for OP-1 in an ELISA, as follows.

1 $\mu\text{g}/100 \mu\text{l}$ of affinity-purified polyclonal rabbit IgG specific for OP-1 is added to each well of a 96-well plate and incubated at 37°C for an hour. The wells are washed four times with 0.167M sodium borate buffer with 0.15 M NaCl (BSB), pH 8.2, containing 0.1% Tween 20. To minimize non-specific binding, the wells are blocked by filling completely with 1% bovine serum albumin (BSA) in BSB and incubating for 1 hour at 37°C . The wells are then washed four times with BSB containing 0.1% Tween 20. A 100 μl aliquot of an appropriate dilution of each of the test samples of cell culture supernatant is added to each well in triplicate and incubated at 37°C for 30 min. After incubation, 100 μl biotinylated rabbit anti-OP-1 serum (stock solution is about 1 mg/ml and diluted 1:400 in BSB containing 1% BSA before use) is added to each well and incubated at 37°C for 30 min. The wells are then washed four times with BSB containing 0.1% Tween 20. 100 μl strepavidin-alkaline (Southern Biotechnology Associates, Inc. Birmingham, Alabama, diluted 1:2000 in

adjuvant and is given subcutaneously. The second injection contains 50 μ g of OP-1 in incomplete adjuvant and is given intraperitoneally. The mouse then receives a total of 230 μ g of OP-1 (amino acids 307-431 in SEQ ID NO:5) in four intraperitoneal injections at various times over an eight month period. One week prior to fusion, both mice are boosted intraperitoneally with 100 μ g of OP-1 (307-431) and 30 μ g of the N-terminal peptide (Ser₂₉₃-Asn₃₀₉-Cys) conjugated through the added cysteine to bovine serum albumin with SMCC crosslinking agent. This boost was repeated five days (IP), four days (IP), three days (IP) and one day (IV) prior to fusion. The mouse spleen cells are then fused to myeloma (e.g., 653) cells at a ratio of 1:1 using PEG 1500 (Boeringer Mannheim), and the cell fusion is plated and screened for OP-1-specific antibodies using OP-1 (307-431) as antigen. The cell fusion and monoclonal screening then are according to standard procedures well described in standard texts widely available in the art.

The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The present embodiments are therefore to be considered in all respects as illustrative and not restrictive, the scope of the invention being indicated by the appended claims rather than by the foregoing description, and all changes which come within the meaning and range of equivalency of the claims are therefore intended to be embraced therein.

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The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The present embodiments are therefore to be considered in all respects as illustrative and not restrictive, the scope of the invention being indicated by the appended claims rather than by the foregoing description, and all changes which come within the meaning and range of equivalency of the claims are therefore intended to be embraced therein.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: RUEGER, DAVID C.
KUBERASAMPATH, THANGAVEL
OPPERMANN, HERMANN
OZKAYNAK ENGIN
PANG, ROY H.L.
COHEN, CHARLES M.
- (ii) TITLE OF INVENTION: MORPHOGEN-INDUCED NERVE REGENERATION AND REPAIR
- (iii) NUMBER OF SEQUENCES: 33
- (iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: TESTA, HURWITZ & THIBEAULT
(B) STREET: 53 STATE STREET
(C) CITY: BOSTON
(D) STATE: MASSACHUSETTS
(E) COUNTRY: U.S.A.
(F) ZIP: 02109
- (v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: Patent In Release #1.0, Version #1.25
- (vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 667,274
(B) FILING DATE: 11-MAR-1991
- (vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 752,764
(B) FILING DATE:

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 97 amino acids
(B) TYPE: amino acids
(C) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (ix) FEATURE:
(A) NAME: Generic Sequence 1

263360" 9344660

(D) OTHER INFORMATION: Each Xaa indicates one of the 20 naturally-occurring L-isomer, α -amino acids or a derivative thereof.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Xaa Xaa Xaa Xaa Xaa Xaa
1 5
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
10 15
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa
20 25
Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
30 35
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
40 45 50
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
55 60
Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
65 70
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
75 80
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
85 90
Xaa Cys Xaa
95

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 97 amino acids

(B) TYPE: amino acids

(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME: Generic Sequence 2

(D) OTHER INFORMATION: Each Xaa indicates one of the 20 naturally-occurring L-isomer, α -amino acids or a derivative thereof.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa				
	1						5			
Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
			10					15		
Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Cys	Xaa	Xaa	Xaa
			20					25		
Cys	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Cys	Xaa	Xaa	Xaa
			30					35		
Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
40						45				50
Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Cys
						55				60
Cys	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
						65				70
Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
						75				80
Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Cys
						85				90
Xaa	Cys	Xaa								
										95

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 97 amino acids

(B) TYPE: amino acids

(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

46360"9346680

(ix) FEATURE:

(A) NAME: Generic Sequence 3

(D) OTHER INFORMATION: wherein each
Xaa is independently selected from
a group of one or more specified
amino acids as defined in the
specification.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```

      Leu Tyr Val Xaa Phe
        1             5
Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa
              10
Xaa Ala Pro Gly Xaa Xaa Xaa Ala
  15             20
Xaa Tyr Cys Xaa Gly Xaa Cys Xaa
          25             30
Xaa Pro Xaa Xaa Xaa Xaa Xaa
              35
Xaa Xaa Xaa Asn His Ala Xaa Xaa
          40             45
Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa
              50
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
          55             60
Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa
              65
Xaa Xaa Xaa Leu Xaa Xaa Xaa
  70             75
Xaa Xaa Xaa Xaa Val Xaa Leu Xaa
              80
Xaa Xaa Xaa Xaa Met Xaa Val Xaa
  85             90
```

463350" 9642680

Xaa Cys Gly Cys Xaa

95

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 102 amino acids

(B) TYPE: amino acids

(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME: Generic Sequence 4

(D) OTHER INFORMATION: wherein each
Xaa is independently selected from
a group of one or more specified
amino acids as defined in the
specification.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Cys Xaa Xaa Xaa Xaa Leu Tyr Val Xaa Phe

1 5 10

Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa

15

Xaa Ala Pro Xaa Gly Xaa Xaa Ala

20 25

Xaa Tyr Cys Xaa Gly Xaa Cys Xaa

30 35

Xaa Pro Xaa Xaa Xaa Xaa Xaa

40

Asn Xaa Xaa Asn His Ala Xaa Xaa

45 50

Xaa Xaa Leu Xaa Xaa Xaa Xaa

55

08937156.08997

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
60 65
Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa
70
Xaa Xaa Xaa Leu Xaa Xaa Xaa
75 80
Xaa Xaa Xaa Xaa Val Xaa Leu Xaa
85
Xaa Xaa Xaa Xaa Met Xaa Val Xaa
90 95
Xaa Cys Gly Cys Xaa
100

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 139 amino acids

(B) TYPE: amino acids

(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME: hOP-1 (mature form)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Ser	Thr	Gly	Ser	Lys	Gln	Arg	Ser	Gln
1				5				
Asn	Arg	Ser	Lys	Thr	Pro	Lys	Asn	Gln
10					15			
Glu	Ala	Leu	Arg	Met	Ala	Asn	Val	Ala
	20					25		
Glu	Asn	Ser	Ser	Ser	Asp	Gln	Arg	Gln
		30					35	
Ala	Cys	Lys	Lys	His	Glu	Leu	Tyr	Val
			40					45
Ser	Phe	Arg	Asp	Leu	Gly	Trp	Gln	Asp
				50				

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Trp	Ile	Ile	Ala	Pro	Glu	Gly	Tyr	Ala
55					60			
Ala	Tyr	Tyr	Cys	Glu	Gly	Glu	Cys	Ala
65					70			
Phe	Pro	Leu	Asn	Ser	Tyr	Met	Asn	Ala
		75					80	
Thr	Asn	His	Ala	Ile	Val	Gln	Thr	Leu
			85					90
Val	His	Phe	Ile	Asn	Pro	Glu	Thr	Val
				95				
Pro	Lys	Pro	Cys	Cys	Ala	Pro	Thr	Gln
100					105			
Leu	Asn	Ala	Ile	Ser	Val	Leu	Tyr	Phe
	110					115		
Asp	Asp	Ser	Ser	Asn	Val	Ile	Leu	Lys
		120					125	
Lys	Tyr	Arg	Asn	Met	Val	Val	Arg	Ala
			130					135
Cys	Gly	Cys	His					

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 139 amino acids

(B) TYPE: amino acids

(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME: mOP-1 (mature form)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Ser	Thr	Gly	Gly	Lys	Gln	Arg	Ser	Gln
1				5				

Asn	Arg	Ser	Lys	Thr	Pro	Lys	Asn	Gln
10					15			
Glu	Ala	Leu	Arg	Met	Ala	Ser	Val	Ala
	20					25		
Glu	Asn	Ser	Ser	Ser	Asp	Gln	Arg	Gln
		30					35	
Ala	Cys	Lys	Lys	His	Glu	Leu	Tyr	Val
			40					45
Ser	Phe	Arg	Asp	Leu	Gly	Trp	Gln	Asp
			50					
Trp	Ile	Ile	Ala	Pro	Glu	Gly	Tyr	Ala
55					60			
Ala	Tyr	Tyr	Cys	Glu	Gly	Glu	Cys	Ala
	65					70		
Phe	Pro	Leu	Asn	Ser	Tyr	Met	Asn	Ala
		75					80	
Thr	Asn	His	Ala	Ile	Val	Gln	Thr	Leu
			85					90
Val	His	Phe	Ile	Asn	Pro	Asp	Thr	Val
				95				
Pro	Lys	Pro	Cys	Cys	Ala	Pro	Thr	Gln
100					105			
Leu	Asn	Ala	Ile	Ser	Val	Leu	Tyr	Phe
	110					115		
Asp	Asp	Ser	Ser	Asn	Val	Ile	Leu	Lys
		120					125	
Lys	Tyr	Arg	Asn	Met	Val	Val	Arg	Ala
			130					135
Cys	Gly	Cys	His					

- (2) INFORMATION FOR SEQ ID NO:7:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 139 amino acids
- (B) TYPE: amino acids
- (C) TOPOLOGY: linear

Ala 1	Val	Arg	Pro	Leu 5	Arg	Arg	Arg	Gln
Pro 10	Lys	Lys	Ser	Asn	Glu 15	Leu	Pro	Gln
Ala 20	Asn	Arg	Leu	Pro	Gly	Ile 25	Phe	Asp
Asp	Val	His 30	Gly	Ser	His	Gly	Arg 35	Gln
Val	Cys	Arg	Arg 40	His	Glu	Leu	Tyr	Val 45
Ser	Phe	Gln	Asp 50	Leu	Gly	Trp	Leu	Asp
Trp 55	Val	Ile	Ala	Pro	Gln 60	Gly	Tyr	Ser
Ala 65	Tyr	Tyr	Cys	Glu	Gly	Glu 70	Cys	Ser
Phe	Pro	Leu 75	Asp	Ser	Cys	Met	Asn 80	Ala
Thr	Asn	His	Ala 85	Ile	Leu	Gln	Ser	Leu 90
Val	His	Leu	Met 95	Lys	Pro	Asn	Ala	Val
Pro 100	Lys	Ala	Cys	Cys	Ala 105	Pro	Thr	Lys
Leu	Ser 110	Ala	Thr	Ser	Val	Leu 115	Tyr	Tyr
Asp	Ser	Ser 120	Asn	Asn	Val	Ile	Leu 125	Arg
Lys	His	Arg	Asn 130	Met	Val	Val	Lys	Ala 135
Cys	Gly	Cys	His					

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 139 amino acids

(B) TYPE: amino acids

(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME: mOP-2 (mature form)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Ala	Ala	Arg	Pro	Leu	Lys	Arg	Arg	Gln	
1				5					
Pro	Lys	Lys	Thr	Asn	Glu	Leu	Pro	His	
10					15				
Pro	Asn	Lys	Leu	Pro	Gly	Ile	Phe	Asp	
	20					25			
Asp	Gly	His	Gly	Ser	Arg	Gly	Arg	Glu	
		30					35		
Val	Cys	Arg	Arg	His	Glu	Leu	Tyr	Val	
			40					45	
Ser	Phe	Arg	Asp	Leu	Gly	Trp	Leu	Asp	
				50					
Trp	Val	Ile	Ala	Pro	Gln	Gly	Tyr	Ser	
55					60				
Ala	Tyr	Tyr	Cys	Glu	Gly	Glu	Cys	Ala	
	65					70			
Phe	Pro	Leu	Asp	Ser	Cys	Met	Asn	Ala	
		75					80		
Thr	Asn	His	Ala	Ile	Leu	Gln	Ser	Leu	
			85					90	
Val	His	Leu	Met	Lys	Pro	Asp	Val	Val	
				95					
Pro	Lys	Ala	Cys	Cys	Ala	Pro	Thr	Lys	
100					105				

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Leu	Ser	Ala	Thr	Ser	Val	Leu	Tyr	Tyr
	110					115		
Asp	Ser	Ser	Asn	Asn	Val	Ile	Leu	Arg
		120					125	
Lys	His	Arg	Asn	Met	Val	Val	Lys	Ala
			130					135
Cys	Gly	Cys	His					

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 96 amino acids

(B) TYPE: amino acids

(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME: CBMP-2A(fx)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Cys	Lys	Arg	His	Pro	Leu	Tyr	Val	Asp	Phe	Ser
1				5					10	
Asp	Val	Gly	Trp	Asn	Asp	Trp	Ile	Val	Ala	Pro
			15						20	
Pro	Gly	Tyr	His	Ala	Phe	Tyr	Cys	His	Gly	Glu
			25						30	
Cys	Pro	Phe	Pro	Leu	Ala	Asp	His	Leu	Asn	Ser
			35						40	
Thr	Asn	His	Ala	Ile	Val	Gln	Thr	Leu	Val	Asn
			45				50			55
Ser	Val	Asn	Ser	Lys	Ile	Pro	Lys	Ala	Cys	Cys
				60						65

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```

Val Pro Thr Glu Leu Ser Ala Ile Ser Met Leu
              70                      75
Tyr Leu Asp Glu Asn Glu Lys Val Val Leu Lys
              80                      85
Asn Tyr Gln Asp Met Val Val Glu Gly Cys Gly
              90                      95
Cys Arg
100

```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 101 amino acids
- (B) TYPE: amino acids
- (C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- (A) NAME: CBMP-2B(fx)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```

                                Cys Arg Arg His Ser
                                1                      5
Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn
                                10                      15
Asp Trp Ile Val Ala Pro Pro Gly Tyr Gln Ala
                                20                      25
Phe Tyr Cys His Gly Asp Cys Pro Phe Pro Leu
                                30                      35
Ala Asp His Leu Asn Ser Thr Asn His Ala Ile
                                40                      45
Val Gln Thr Leu Val Asn Ser Val Asn Ser Ser
                                50                      55                      60
Ile Pro Lys Ala Cys Cys Val Pro Thr Glu Leu
                                65                      70

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Ser Ala Ile Ser Met Leu Tyr Leu Asp Glu Tyr
75 80
Asp Lys Val Val Leu Lys Asn Tyr Gln Glu Met
85 90
Val Val Glu Gly Cys Gly Cys Arg
95 100

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 102 amino acids

(B) TYPE: amino acids

(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME: DPP(fx)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Cys Arg Arg His Ser Leu Tyr Val Asp Phe Ser
1 5 10
Asp Val Gly Trp Asp Asp Trp Ile Val Ala Pro
15 20
Leu Gly Tyr Asp Ala Tyr Tyr Cys His Gly Lys
25 30
Cys Pro Phe Pro Leu Ala Asp His Phe Asn Ser
35 40
Thr Asn His Ala Val Val Gln Thr Leu Val Asn
45 50 55
Asn Asn Asn Pro Gly Lys Val Pro Lys Ala Cys
60 65
Cys Val Pro Thr Gln Leu Asp Ser Val Ala Met
70 75

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Leu Tyr Leu Asn Asp Gln Ser Thr Val Val Leu
 80 85
 Lys Asn Tyr Gln Glu Met Thr Val Val Gly Cys
 90 95
 Gly Cys Arg
 100

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 102 amino acids

(B) TYPE: amino acids

(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME: Vgl(fx)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Cys Lys Lys Arg His Leu Tyr Val Glu Phe Lys
 1 5 10
 Asp Val Gly Trp Gln Asn Trp Val Ile Ala Pro
 15 20
 Gln Gly Tyr Met Ala Asn Tyr Cys Tyr Gly Glu
 25 30
 Cys Pro Tyr Pro Leu Thr Glu Ile Leu Asn Gly
 35 40
 Ser Asn His Ala Ile Leu Gln Thr Leu Val His
 45 50 55
 Ser Ile Glu Pro Glu Asp Ile Pro Leu Pro Cys
 60 65
 Cys Val Pro Thr Lys Met Ser Pro Ile Ser Met
 70 75

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 26350 99/2680

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 102 amino acids

(B) TYPE: amino acids

(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME: Vgr-1(fx)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Cys Lys Lys His Glu Leu Tyr Val Ser Phe Gln
1 5 10
Asp Val Gly Trp Gln Asp Trp Ile Ile Ala Pro
15 20
Xaa Gly Tyr Ala Ala Asn Tyr Cys Asp Gly Glu
25 30
Cys Ser Phe Pro Leu Asn Ala His Met Asn Ala
35 40
Thr Asn His Ala Ile Val Gln Thr Leu Val His
45 50 55
Val Met Asn Pro Glu Tyr Val Pro Lys Pro Cys
60 65
Cys Ala Pro Thr Lys Val Asn Ala Ile Ser Val
70 75

Leu Tyr Phe Asp Asp Asn Ser Asn Val Ile Leu
80 85
Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys
90 95
Gly Cys His
100

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 106 amino acids
- (B) TYPE: protein
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein

- (vi) ORIGINAL SOURCE:
- (A) ORGANISM: human
- (F) TISSUE TYPE: BRAIN

- (ix) FEATURE:
- (D) OTHER INFORMATION:
- /product= "GDF-1 (fx)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Cys Arg Ala Arg Arg Leu Tyr Val Ser Phe Arg Glu Val Gly
1 5 10
Trp His Arg Trp Val Ile Ala Pro Arg Gly Phe Leu Ala Asn Tyr
15 20 25
Cys Gln Gly Gln Cys Ala Leu Pro Val Ala Leu Ser Gly Ser Gly
30 35 40
Gly Pro Pro Ala Leu Asn His Ala Val Leu Arg Ala Leu Met His
45 50 55
Ala Ala Ala Pro Gly Ala Ala Asp Leu Pro Cys Cys Val Pro Ala
60 65 70
Arg Leu Ser Pro Ile Ser Val Leu Phe Phe Asp Asn Ser Asp Asn
75 80 85
Val Val Leu Arg Gln Tyr Glu Asp Met Val Val Asp Glu Cys Gly
90 95 100
Cys Arg
105

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(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 5 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Cys Xaa Xaa Xaa Xaa
1 5

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1822 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: HOMO SAPIENS
- (F) TISSUE TYPE: HIPPOCAMPUS

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 49..1341
- (D) OTHER INFORMATION: /standard_name= "hOP1"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GGTGCGGGCC CGGAGCCCGG AGCCCGGGTA GCGCGTAGAG CCGGCGCG	ATG CAC GTG	57
	Met His Val	
	1	
CGC TCA CTG CGA GCT GCG GCG CCG CAC AGC TTC GTG GCG CTC TGG GCA		105
Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala Leu Trp Ala		
5 10 15		
CCC CTG TTC CTG CTG CGC TCC GCC CTG GCC GAC TTC AGC CTG GAC AAC		153
Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser Leu Asp Asn		
20 25 30 35		
GAG GTG CAC TCG AGC TTC ATC CAC CGG CGC CTC CGC AGC CAG GAG CGG		201
Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser Gln Glu Arg		
40 45 50		

CGG	GAG	ATG	CAG	CGC	GAG	ATC	CTC	TCC	ATT	TTG	GGC	TTG	CCC	CAC	CGC	249
Arg	Glu	Met	Gln	Arg	Glu	Ile	Leu	Ser	Ile	Leu	Gly	Leu	Pro	His	Arg	
			55					60					65			
CCG	CGC	CCG	CAC	CTC	CAG	GGC	AAG	CAC	AAC	TCG	GCA	CCC	ATG	TTC	ATG	297
Pro	Arg	Pro	His	Leu	Gln	Gly	Lys	His	Asn	Ser	Ala	Pro	Met	Phe	Met	
		70					75					80				
CTG	GAC	CTG	TAC	AAC	GCC	ATG	GCG	GTG	GAG	GAG	GGC	GGC	GGG	CCC	GGC	345
Leu	Asp	Leu	Tyr	Asn	Ala	Met	Ala	Val	Glu	Glu	Gly	Gly	Gly	Pro	Gly	
	85					90					95					
GGC	CAG	GGC	TTC	TCC	TAC	CCC	TAC	AAG	GCC	GTC	TTC	AGT	ACC	CAG	GGC	393
Gly	Gln	Gly	Phe	Ser	Tyr	Pro	Tyr	Lys	Ala	Val	Phe	Ser	Thr	Gln	Gly	
100					105					110					115	
CCC	CCT	CTG	GCC	AGC	CTG	CAA	GAT	AGC	CAT	TTC	CTC	ACC	GAC	GCC	GAC	441
Pro	Pro	Leu	Ala	Ser	Leu	Gln	Asp	Ser	His	Phe	Leu	Thr	Asp	Ala	Asp	
				120				125						130		
ATG	GTC	ATG	AGC	TTC	GTC	AAC	CTC	GTG	GAA	CAT	GAC	AAG	GAA	TTC	TTC	489
Met	Val	Met	Ser	Phe	Val	Asn	Leu	Val	Glu	His	Asp	Lys	Glu	Phe	Phe	
			135					140					145			
CAC	CCA	CGC	TAC	CAC	CAT	CGA	GAG	TTC	CGG	TTT	GAT	CTT	TCC	AAG	ATC	537
His	Pro	Arg	Tyr	His	His	Arg	Glu	Phe	Arg	Phe	Asp	Leu	Ser	Lys	Ile	
		150					155					160				
CCA	GAA	GGG	GAA	GCT	GTC	ACG	GCA	GCC	GAA	TTC	CGG	ATC	TAC	AAG	GAC	585
Pro	Glu	Gly	Glu	Ala	Val	Thr	Ala	Ala	Glu	Phe	Arg	Ile	Tyr	Lys	Asp	
	165					170					175					
TAC	ATC	CGG	GAA	CGC	TTC	GAC	AAT	GAG	ACG	TTC	CGG	ATC	AGC	GTT	TAT	633
Tyr	Ile	Arg	Glu	Arg	Phe	Asp	Asn	Glu	Thr	Phe	Arg	Ile	Ser	Val	Tyr	
180					185					190					195	
CAG	GTG	CTC	CAG	GAG	CAC	TTG	GGC	AGG	GAA	TCG	GAT	CTC	TTC	CTG	CTC	681
Gln	Val	Leu	Gln	Glu	His	Leu	Gly	Arg	Glu	Ser	Asp	Leu	Phe	Leu	Leu	
				200				205						210		
GAC	AGC	CGT	ACC	CTC	TGG	GCC	TCG	GAG	GAG	GGC	TGG	CTG	GTG	TTT	GAC	729
Asp	Ser	Arg	Thr	Leu	Trp	Ala	Ser	Glu	Glu	Gly	Trp	Leu	Val	Phe	Asp	
			215					220					225			
ATC	ACA	GCC	ACC	AGC	AAC	CAC	TGG	GTG	GTC	AAT	CCG	CGG	CAC	AAC	CTG	777
Ile	Thr	Ala	Thr	Ser	Asn	His	Trp	Val	Val	Asn	Pro	Arg	His	Asn	Leu	
		230					235					240				
GGC	CTG	CAG	CTC	TCG	GTG	GAG	ACG	CTG	GAT	GGG	CAG	AGC	ATC	AAC	CCC	825
Gly	Leu	Gln	Leu	Ser	Val	Glu	Thr	Leu	Asp	Gly	Gln	Ser	Ile	Asn	Pro	
	245					250					255					

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[illegible]

GCATAAAGAA AAATGGCCGG GCCAGGTCAT TGGCTGGGAA GTCTCAGCCA TGCACGGACT 1651
CGTTTCCAGA GGTAATTATG AGCGCCTACC AGCCAGGCCA CCCAGCCGTG GGAGGAAGGG 1711
GGCGTGGCAA GGGGTGGGCA CATTGGTGTC TGTGCGAAAG GAAAATTGAC CCGGAAGTTC 1771
CTGTAATAAA TGTACAATA AAACGAATGA ATGAAAAAAAA AAAAAAAAAA A 1822

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 431 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(D) OTHER INFORMATION: /Product="OP1-PP"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Met	His	Val	Arg	Ser	Leu	Arg	Ala	Ala	Ala	Pro	His	Ser	Phe	Val	Ala	
1				5					10					15		
Leu	Trp	Ala	Pro	Leu	Phe	Leu	Leu	Arg	Ser	Ala	Leu	Ala	Asp	Phe	Ser	
			20					25					30			
Leu	Asp	Asn	Glu	Val	His	Ser	Ser	Phe	Ile	His	Arg	Arg	Leu	Arg	Ser	
		35					40					45				
Gln	Glu	Arg	Arg	Glu	Met	Gln	Arg	Glu	Ile	Leu	Ser	Ile	Leu	Gly	Leu	
	50					55					60					
Pro	His	Arg	Pro	Arg	Pro	His	Leu	Gln	Gly	Lys	His	Asn	Ser	Ala	Pro	
65					70					75					80	
Met	Phe	Met	Leu	Asp	Leu	Tyr	Asn	Ala	Met	Ala	Val	Glu	Glu	Gly	Gly	
			85						90					95		
Gly	Pro	Gly	Gly	Gln	Gly	Phe	Ser	Tyr	Pro	Tyr	Lys	Ala	Val	Phe	Ser	
		100						105					110			
Thr	Gln	Gly	Pro	Pro	Leu	Ala	Ser	Leu	Gln	Asp	Ser	His	Phe	Leu	Thr	
		115					120					125				
Asp	Ala	Asp	Met	Val	Met	Ser	Phe	Val	Asn	Leu	Val	Glu	His	Asp	Lys	
	130					135					140					
Glu	Phe	Phe	His	Pro	Arg	Tyr	His	His	Arg	Glu	Phe	Arg	Phe	Asp	Leu	
145					150				155						160	

Ser Lys Ile Pro Glu Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile
165 170 175

Tyr Lys Asp Tyr Ile Arg Glu Arg Phe Asp Asn Glu Thr Phe Arg Ile
180 185 190

Ser Val Tyr Gln Val Leu Gln Glu His Leu Gly Arg Glu Ser Asp Leu
195 200 205

Phe Leu Leu Asp Ser Arg Thr Leu Trp Ala Ser Glu Glu Gly Trp Leu
210 215 220

Val Phe Asp Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg
225 230 235 240

His Asn Leu Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser
245 250 255

Ile Asn Pro Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn
260 265 270

Lys Gln Pro Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Phe
275 280 285

Arg Ser Ile Arg Ser Thr Gly Ser Lys Gln Arg Ser Gln Asn Arg Ser
290 295 300

Lys Thr Pro Lys Asn Gln Glu Ala Leu Arg Met Ala Asn Val Ala Glu
305 310 315 320

Asn Ser Ser Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr
325 330 335

Val Ser Phe Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu
340 345 350

Gly Tyr Ala Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn
355 360 365

Ser Tyr Met Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His
370 375 380

Phe Ile Asn Pro Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln
385 390 395 400

Leu Asn Ala Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile
405 410 415

Leu Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His
420 425 430

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(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1873 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: MURIDAE
- (F) TISSUE TYPE: EMBRYO

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 104..1393
- (D) OTHER INFORMATION: /note= "MOP1 (CDNA)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CTGCAGCAAG TGACCTCGGG TCGTGGACCG CTGCCCTGCC CCCTCCGCTG CCACCTGGGG	60
CGGCGCGGGC CCGGTGCCCC GGATCGCGCG TAGAGCCGGC GCG ATG CAC GTG CGC	115
Met His Val Arg	
1	
TCG CTG CGC GCT GCG GCG CCA CAC AGC TTC GTG GCG CTC TGG GCG CCT	163
Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala Leu Trp Ala Pro	
5 10 15 20	
CTG TTC TTG CTG CGC TCC GCC CTG GCC GAT TTC AGC CTG GAC AAC GAG	211
Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser Leu Asp Asn Glu	
25 30 35	
GTG CAC TCC AGC TTC ATC CAC CGG CGC CTC CGC AGC CAG GAG CGG CGG	259
Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser Gln Glu Arg Arg	
40 45 50	
GAG ATG CAG CGG GAG ATC CTG TCC ATC TTA GGG TTG CCC CAT CGC CCG	307
Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu Pro His Arg Pro	
55 60 65	
CGC CCG CAC CTC CAG GGA AAG CAT AAT TCG GCG CCC ATG TTC ATG TTG	355
Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro Met Phe Met Leu	
70 75 80	
GAC CTG TAC AAC GCC ATG GCG GTG GAG GAG AGC GGG CCG GAC GGA CAG	403
Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Ser Gly Pro Asp Gly Gln	
85 90 95 100	

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GGC	TTC	TCC	TAC	CCC	TAC	AAG	GCC	GTC	TTC	AGT	ACC	CAG	GGC	CCC	CCT	451
Gly	Phe	Ser	Tyr	Pro	Tyr	Lys	Ala	Val	Phe	Ser	Thr	Gln	Gly	Pro	Pro	
				105					110					115		
TTA	GCC	AGC	CTG	CAG	GAC	AGC	CAT	TTC	CTC	ACT	GAC	GCC	GAC	ATG	GTC	499
Leu	Ala	Ser	Leu	Gln	Asp	Ser	His	Phe	Leu	Thr	Asp	Ala	Asp	Met	Val	
			120					125					130			
ATG	AGC	TTC	GTC	AAC	CTA	GTG	GAA	CAT	GAC	AAA	GAA	TTC	TTC	CAC	CCT	547
Met	Ser	Phe	Val	Asn	Leu	Val	Glu	His	Asp	Lys	Glu	Phe	Phe	His	Pro	
		135					140					145				
CGA	TAC	CAC	CAT	CGG	GAG	TTC	CGG	TTT	GAT	CTT	TCC	AAG	ATC	CCC	GAG	595
Arg	Tyr	His	His	Arg	Glu	Phe	Arg	Phe	Asp	Leu	Ser	Lys	Ile	Pro	Glu	
	150					155					160					
GGC	GAA	CGG	GTG	ACC	GCA	GCC	GAA	TTC	AGG	ATC	TAT	AAG	GAC	TAC	ATC	643
Gly	Glu	Arg	Val	Thr	Ala	Ala	Glu	Phe	Arg	Ile	Tyr	Lys	Asp	Tyr	Ile	
165					170				175						180	
CGG	GAG	CGA	TTT	GAC	AAC	GAG	ACC	TTC	CAG	ATC	ACA	GTC	TAT	CAG	GTG	691
Arg	Glu	Arg	Phe	Asp	Asn	Glu	Thr	Phe	Gln	Ile	Thr	Val	Tyr	Gln	Val	
				185					190					195		
CTC	CAG	GAG	CAC	TCA	GGC	AGG	GAG	TCG	GAC	CTC	TTC	TTG	CTG	GAC	AGC	739
Leu	Gln	Glu	His	Ser	Gly	Arg	Glu	Ser	Asp	Leu	Phe	Leu	Leu	Asp	Ser	
			200					205					210			
CGC	ACC	ATC	TGG	GCT	TCT	GAG	GAG	GGC	TGG	TTG	GTG	TTT	GAT	ATC	ACA	787
Arg	Thr	Ile	Trp	Ala	Ser	Glu	Glu	Gly	Trp	Leu	Val	Phe	Asp	Ile	Thr	
		215					220					225				
GCC	ACC	AGC	AAC	CAC	TGG	GTG	GTC	AAC	CCT	CGG	CAC	AAC	CTG	GGC	TTA	835
Ala	Thr	Ser	Asn	His	Trp	Val	Val	Asn	Pro	Arg	His	Asn	Leu	Gly	Leu	
	230					235					240					
CAG	CTC	TCT	GTG	GAG	ACC	CTG	GAT	GGG	CAG	AGC	ATC	AAC	CCC	AAG	TTG	883
Gln	Leu	Ser	Val	Glu	Thr	Leu	Asp	Gly	Gln	Ser	Ile	Asn	Pro	Lys	Leu	
245					250					255					260	
GCA	GGC	CTG	ATT	GGA	CGG	CAT	GGA	CCC	CAG	AAC	AAG	CAA	CCC	TTC	ATG	931
Ala	Gly	Leu	Ile	Gly	Arg	His	Gly	Pro	Gln	Asn	Lys	Gln	Pro	Phe	Met	
				265				270						275		
GTG	GCC	TTC	TTC	AAG	GCC	ACG	GAA	GTC	CAT	CTC	CGT	AGT	ATC	CGG	TCC	979
Val	Ala	Phe	Phe	Lys	Ala	Thr	Glu	Val	His	Leu	Arg	Ser	Ile	Arg	Ser	
			280					285					290			
ACG	GGG	GGC	AAG	CAG	CGC	AGC	CAG	AAT	CGC	TCC	AAG	ACG	CCA	AAG	AAC	1027
Thr	Gly	Gly	Lys	Gln	Arg	Ser	Gln	Asn	Arg	Ser	Lys	Thr	Pro	Lys	Asn	
		295					300					305				

451 499 547 595 643 691 739 787 835 883 931 979 1027

CAA GAG GCC CTG AGG ATG GCC AGT GTG GCA GAA AAC AGC AGC AGT GAC	1075
Gln Glu Ala Leu Arg Met Ala Ser Val Ala Glu Asn Ser Ser Ser Asp	
310 315 320	
CAG AGG CAG GCC TGC AAG AAA CAT GAG CTG TAC GTC AGC TTC CGA GAC	1123
Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg Asp	
325 330 335 340	
CTT GGC TGG CAG GAC TGG ATC ATT GCA CCT GAA GGC TAT GCT GCC TAC	1171
Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala Tyr	
345 350 355	
TAC TGT GAG GGA GAG TGC GCC TTC CCT CTG AAC TCC TAC ATG AAC GCC	1219
Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met Asn Ala	
360 365 370	
ACC AAC CAC GCC ATC GTC CAG ACA CTG GTT CAC TTC ATC AAC CCA GAC	1267
Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn Pro Asp	
375 380 385	
ACA GTA CCC AAG CCC TGC TGT GCG CCC ACC CAG CTC AAC GCC ATC TCT	1315
Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala Ile Ser	
390 395 400	
GTC CTC TAC TTC GAC GAC AGC TCT AAT GTC ATC CTG AAG AAG TAC AGA	1363
Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr Arg	
405 410 415 420	
AAC ATG GTG GTC CGG GCC TGT GGC TGC CAC TAGCTCTTCC TGAGACCCTG	1413
Asn Met Val Val Arg Ala Cys Gly Cys His	
425 430	
ACCTTTGCGG GGCCACACCT TTCCAAATCT TCGATGTCTC ACCATCTAAG TCTCTCACTG	1473
CCCACCTTGG CGAGGAGAAC AGACCAACCT CTCCTGAGCC TTCCCTCACC TCCCAACCGG	1533
AAGCATGTAA GGGTTCCAGA AACCTGAGCG TGCAGCAGCT GATGAGCGCC CTTTCCTTCT	1593
GGCACGTGAC GGACAAGATC CTACCAGCTA CCACAGCAAA CGCCTAAGAG CAGGAAAAAT	1653
GTCTGCCAGG AAAGTGTCCA GTGTCCACAT GGCCCCTGGC GCTCTGAGTC TTTGAGGAGT	1713
AATCGCAAGC CTCGTTGAGC TGCAGCAGAA GGAAGGGCTT AGCCAGGGTG GGCGCTGGCG	1773
TCTGTGTTGA AGGGAAACCA AGCAGAAGCC ACTGTAATGA TATGTCACAA TAAAACCCAT	1833
GAATGAAAAA AAAAAAAAAA AAAAAAAAAA AAAAGAATTC	1873

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 430 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(D) OTHER INFORMATION: /product= "mOP1-PP"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Met His Val Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala
1 5 10 15
Leu Trp Ala Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser
20 25 30
Leu Asp Asn Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser
35 40 45
Gln Glu Arg Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu
50 55 60
Pro His Arg Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro
65 70 75 80
Met Phe Met Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Ser Gly
85 90 95
Pro Asp Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser Thr
100 105 110
Gln Gly Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr Asp
115 120 125
Ala Asp Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys Glu
130 135 140
Phe Phe His Pro Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu Ser
145 150 155 160
Lys Ile Pro Glu Gly Glu Arg Val Thr Ala Ala Glu Phe Arg Ile Tyr
165 170 175
Lys Asp Tyr Ile Arg Glu Arg Phe Asp Asn Glu Thr Phe Gln Ile Thr
180 185 190
Val Tyr Gln Val Leu Gln Glu His Ser Gly Arg Glu Ser Asp Leu Phe
195 200 205

Leu Leu Asp Ser Arg Thr Ile Trp Ala Ser Glu Glu Gly Trp Leu Val
 210 215 220
 Phe Asp Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg His
 225 230 235 240
 Asn Leu Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser Ile
 245 250 255
 Asn Pro Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn Lys
 260 265 270
 Gln Pro Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Leu Arg
 275 280 285
 Ser Ile Arg Ser Thr Gly Gly Lys Gln Arg Ser Gln Asn Arg Ser Lys
 290 295 300
 Thr Pro Lys Asn Gln Glu Ala Leu Arg Met Ala Ser Val Ala Glu Asn
 305 310 315 320
 Ser Ser Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val
 325 330 335
 Ser Phe Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly
 340 345 350
 Tyr Ala Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser
 355 360 365
 Tyr Met Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe
 370 375 380
 Ile Asn Pro Asp Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu
 385 390 395 400
 Asn Ala Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu
 405 410 415
 Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His
 420 425 430

(2) INFORMATION FOR SEQ ID NO:20:

(i)SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1723 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii)MOLECULE TYPE: cDNA

269260"93446680

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens
(F) TISSUE TYPE: HIPPOCAMPUS

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 490..1696
(D) OTHER INFORMATION: /note= "hOP2 (cDNA)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GGCGCCGGCA GAGCAGGAGT GGCTGGAGGA GCTGTGGTTG GAGCAGGAGG TGGCACGGCA	60
GGGCTGGAGG GCTCCCTATG AGTGGCGGAG ACGGCCAGG AGGCGCTGGA GCAACAGCTC	120
CCACACCGCA CCAAGCGGTG GCTGCAGGAG CTCGCCCATC GCCCCTGCGC TGCTCGGACC	180
GCGGCCACAG CCGGACTGGC GGGTACGGCG GCGACAGAGG CATTGGCCGA GAGTCCCAGT	240
CCGCAGAGTA GCCCCGGCCT CGAGGCGGTG GCGTCCCGGT CCTCTCCGTC CAGGAGCCAG	300
GACAGGTGTC GCGCGGCGGG GCTCCAGGGA CCGCGCCTGA GGCCGGCTGC CCGCCCGTCC	360
CGCCCCGCCC CGCCGCCCCG CGCCCGCCGA GCCCAGCCTC CTTGCCGTGC GGGCGTCCCC	420
AGGCCCTGGG TCGGCCGCGG AGCCGATGCG CGCCCGCTGA GCGCCCCAGC TGAGCGCCCC	480
CGGCCTGCC ATG ACC GCG CTC CCC GGC CCG CTC TGG CTC CTG GGC CTG	528
Met Thr Ala Leu Pro Gly Pro Leu Trp Leu Leu Gly Leu	
1 5 10	
GCG CTA TGC GCG CTG GGC GGG GGC GGC CCC GGC CTG CGA CCC CCG CCC	576
Ala Leu Cys Ala Leu Gly Gly Gly Gly Pro Gly Leu Arg Pro Pro Pro	
15 20 25	
GGC TGT CCC CAG CGA CGT CTG GGC GCG CGC GAG CGC CGG GAC GTG CAG	624
Gly Cys Pro Gln Arg Arg Leu Gly Ala Arg Glu Arg Arg Asp Val Gln	
30 35 40 45	
CGC GAG ATC CTG GCG GTG CTC GGG CTG CCT GGG CGG CCC CGG CCC CGC	672
Arg Glu Ile Leu Ala Val Leu Gly Leu Pro Gly Arg Pro Arg Pro Arg	
50 55 60	
GCG CCA CCC GCC GCC TCC CGG CTG CCC GCG TCC GCG CCG CTC TTC ATG	720
Ala Pro Pro Ala Ala Ser Arg Leu Pro Ala Ser Ala Pro Leu Phe Met	
65 70 75	
CTG GAC CTG TAC CAC GCC ATG GCC GGC GAC GAC GAC GAG GAC GGC GCG	768
Leu Asp Leu Tyr His Ala Met Ala Gly Asp Asp Asp Glu Asp Gly Ala	
80 85 90	

269690" 5346690

CCC Pro	GCG Ala	GAG Glu	CGG Arg	CGC Arg	CTG Leu	GGC Gly	CGC Arg	GCC Ala	GAC Asp	CTG Leu	GTC Val	ATG Met	AGC Ser	TTC Phe	GTT Val	816
	95					100					105					
AAC Asn	ATG Met	GTG Val	GAG Glu	CGA Arg	GAC Asp	CGT Arg	GCC Ala	CTG Leu	GGC Gly	CAC His	CAG Gln	GAG Glu	CCC Pro	CAT His	TGG Trp	864
110						115				120					125	
AAG Lys	GAG Glu	TTC Phe	CGC Arg	TTT Phe	GAC Asp	CTG Leu	ACC Thr	CAG Gln	ATC Ile	CCG Pro	GCT Ala	GGG Gly	GAG Glu	GCG Ala	GTC Val	912
				130					135					140		
ACA Thr	GCT Ala	GCG Ala	GAG Glu	TTC Phe	CGG Arg	ATT Ile	TAC Tyr	AAG Lys	GTG Val	CCC Pro	AGC Ser	ATC Ile	CAC His	CTG Leu	CTC Leu	960
			145					150					155			
AAC Asn	AGG Arg	ACC Thr	CTC Leu	CAC His	GTC Val	AGC Ser	ATG Met	TTC Phe	CAG Gln	GTG Val	GTC Val	CAG Gln	GAG Glu	CAG Gln	TCC Ser	1008
		160					165					170				
AAC Asn	AGG Arg	GAG Glu	TCT Ser	GAC Asp	TTG Leu	TTC Phe	TTT Phe	TTG Leu	GAT Asp	CTT Leu	CAG Gln	ACG Thr	CTC Leu	CGA Arg	GCT Ala	1056
	175					180					185					
GGA Gly	GAC Asp	GAG Glu	GGC Gly	TGG Trp	CTG Leu	GTG Val	CTG Leu	GAT Asp	GTC Val	ACA Thr	GCA Ala	GCC Ala	AGT Ser	GAC Asp	TGC Cys	1104
190					195					200					205	
TGG Trp	TTG Leu	CTG Leu	AAG Lys	CGT Arg	CAC His	AAG Lys	GAC Asp	CTG Leu	GGA Gly	CTC Leu	CGC Arg	CTC Leu	TAT Tyr	GTG Val	GAG Glu	1152
				210					215					220		
ACT Thr	GAG Glu	GAC Asp	GGG Gly	CAC His	AGC Ser	GTG Val	GAT Asp	CCT Pro	GGC Gly	CTG Leu	GCC Ala	GGC Gly	CTG Leu	CTG Leu	GGT Gly	1200
			225					230					235			
CAA Gln	CGG Arg	GCC Ala	CCA Pro	CGC Arg	TCC Ser	CAA Gln	CAG Gln	CCT Pro	TTC Phe	GTG Val	GTC Val	ACT Thr	TTC Phe	TTC Phe	AGG Arg	1248
		240					245					250				
GCC Ala	AGT Ser	CCG Pro	AGT Ser	CCC Pro	ATC Ile	CGC Arg	ACC Thr	CCT Pro	CGG Arg	GCA Ala	GTG Val	AGG Arg	CCA Pro	CTG Leu	AGG Arg	1296
	255					260					265					
AGG Arg	AGG Arg	CAG Gln	CCG Pro	AAG Lys	AAA Lys	AGC Ser	AAC Asn	GAG Glu	CTG Leu	CCG Pro	CAG Gln	GCC Ala	AAC Asn	CGA Arg	CTC Leu	1344
270					275					280					285	
CCA Pro	GGG Gly	ATC Ile	TTT Phe	GAT Asp	GAC Asp	GTC Val	CAC His	GGC Gly	TCC Ser	CAC His	GGC Gly	CGG Arg	CAG Gln	GTC Val	TGC Cys	1392
				290					295					300		

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CGT	CGG	CAC	GAG	CTC	TAC	GTC	AGC	TTC	CAG	GAC	CTC	GGC	TGG	CTG	GAC	1440
Arg	Arg	His	Glu	Leu	Tyr	Val	Ser	Phe	Gln	Asp	Leu	Gly	Trp	Leu	Asp	
			305					310					315			
TGG	GTC	ATC	GCT	CCC	CAA	GGC	TAC	TCG	GCC	TAT	TAC	TGT	GAG	GGG	GAG	1488
Trp	Val	Ile	Ala	Pro	Gln	Gly	Tyr	Ser	Ala	Tyr	Tyr	Cys	Glu	Gly	Glu	
			320				325					330				
TGC	TCC	TTC	CCA	CTG	GAC	TCC	TGC	ATG	AAT	GCC	ACC	AAC	CAC	GCC	ATC	1536
Cys	Ser	Phe	Pro	Leu	Asp	Ser	Cys	Met	Asn	Ala	Thr	Asn	His	Ala	Ile	
			335				340				345					
CTG	CAG	TCC	CTG	GTG	CAC	CTG	ATG	AAG	CCA	AAC	GCA	GTC	CCC	AAG	GCG	1584
Leu	Gln	Ser	Leu	Val	His	Leu	Met	Lys	Pro	Asn	Ala	Val	Pro	Lys	Ala	
					355					360					365	
TGC	TGT	GCA	CCC	ACC	AAG	CTG	AGC	GCC	ACC	TCT	GTG	CTC	TAC	TAT	GAC	1632
Cys	Cys	Ala	Pro	Thr	Lys	Leu	Ser	Ala	Thr	Ser	Val	Leu	Tyr	Tyr	Asp	
					370				375					380		
AGC	AGC	AAC	AAC	GTC	ATC	CTG	CGC	AAA	CAC	CGC	AAC	ATG	GTG	GTC	AAG	1680
Ser	Ser	Asn	Asn	Val	Ile	Leu	Arg	Lys	His	Arg	Asn	Met	Val	Val	Lys	
			385					390					395			
GCC	TGC	GGC	TGC	CAC	T	GAGTCAGCCC	GCCCAGCCCT	ACTGCAG								1723
Ala	Cys	Gly	Cys	His												
			400													

(2) INFORMATION FOR SEQ ID NO:21:

(i)SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 402 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii)MOLECULE TYPE: protein

(ix)FEATURE:

- (A)OTHER INFORMATION: /product= "hOP2-PP"

(xi)SEQUENCE DESCRIPTION: SEQ ID NO:21:

Met	Thr	Ala	Leu	Pro	Gly	Pro	Leu	Trp	Leu	Leu	Gly	Leu	Ala	Leu	Cys
1				5					10					15	
Ala	Leu	Gly	Gly	Gly	Gly	Pro	Gly	Leu	Arg	Pro	Pro	Pro	Gly	Cys	Pro
			20					25					30		
Gln	Arg	Arg	Leu	Gly	Ala	Arg	Glu	Arg	Arg	Asp	Val	Gln	Arg	Glu	Ile
			35				40					45			

Leu Ala Val Leu Gly Leu Pro Gly Arg Pro Arg Pro Arg Ala Pro Pro
 50 55 60
 Ala Ala Ser Arg Leu Pro Ala Ser Ala Pro Leu Phe Met Leu Asp Leu
 65 70 75 80
 Tyr His Ala Met Ala Gly Asp Asp Asp Glu Asp Gly Ala Pro Ala Glu
 85 90 95
 Arg Arg Leu Gly Arg Ala Asp Leu Val Met Ser Phe Val Asn Met Val
 100 105 110
 Glu Arg Asp Arg Ala Leu Gly His Gln Glu Pro His Trp Lys Glu Phe
 115 120 125
 Arg Phe Asp Leu Thr Gln Ile Pro Ala Gly Glu Ala Val Thr Ala Ala
 130 135 140
 Glu Phe Arg Ile Tyr Lys Val Pro Ser Ile His Leu Leu Asn Arg Thr
 145 150 155 160
 Leu His Val Ser Met Phe Gln Val Val Gln Glu Gln Ser Asn Arg Glu
 165 170 175
 Ser Asp Leu Phe Phe Leu Asp Leu Gln Thr Leu Arg Ala Gly Asp Glu
 180 185 190
 Gly Trp Leu Val Leu Asp Val Thr Ala Ala Ser Asp Cys Trp Leu Leu
 195 200 205
 Lys Arg His Lys Asp Leu Gly Leu Arg Leu Tyr Val Glu Thr Glu Asp
 210 215 220
 Gly His Ser Val Asp Pro Gly Leu Ala Gly Leu Leu Gly Gln Arg Ala
 225 230 235 240
 Pro Arg Ser Gln Gln Pro Phe Val Val Thr Phe Phe Arg Ala Ser Pro
 245 250 255
 Ser Pro Ile Arg Thr Pro Arg Ala Val Arg Pro Leu Arg Arg Arg Gln
 260 265 270
 Pro Lys Lys Ser Asn Glu Leu Pro Gln Ala Asn Arg Leu Pro Gly Ile
 275 280 285
 Phe Asp Asp Val His Gly Ser His Gly Arg Gln Val Cys Arg Arg His
 290 295 300
 Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Leu Asp Trp Val Ile
 305 310 315 320

253260" 3542660

Ala Pro Gln Gly Tyr Ser Ala Tyr Tyr Cys Glu Gly Glu Cys Ser Phe
325 330 335

Pro Leu Asp Ser Cys Met Asn Ala Thr Asn His Ala Ile Leu Gln Ser
340 345 350

Leu Val His Leu Met Lys Pro Asn Ala Val Pro Lys Ala Cys Cys Ala
355 360 365

Pro Thr Lys Leu Ser Ala Thr Ser Val Leu Tyr Tyr Asp Ser Ser Asn
370 375 380

Asn Val Ile Leu Arg Lys His Arg Asn Met Val Val Lys Ala Cys Gly
385 390 395 400

Cys His

(2) INFORMATION FOR SEQ ID NO:22:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1926 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: MURIDAE
 - (F) TISSUE TYPE: EMBRYO
- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 93..1289
 - (D) OTHER INFORMATION: /note= "mOP2 cDNA"
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GCCAGGCACA GGTGCGCCGT CTGGTCCTCC CCGTCTGGCG TCAGCCGAGC	50
CCGACCAGCT ACCAGTGGAT GCGCGCCGGC TGAAAGTCCG AG ATG GCT ATG CGT	104
Met Ala Met Arg	
1	
CCC GGG CCA CTC TGG CTA TTG GGC CTT GCT CTG TGC GCG CTG GGA GGC	152
Pro Gly Pro Leu Trp Leu Leu Gly Leu Ala Leu Cys Ala Leu Gly Gly	
5 10 15 20	
GGC CAC GGT CCG CGT CCC CCG CAC ACC TGT CCC CAG CGT CGC CTG GGA	200
Gly His Gly Pro Arg Pro Pro His Thr Cys Pro Gln Arg Arg Leu Gly	
25 30 35	

0993756.09997

GCG CGC GAG CGC CGC GAC ATG CAG CGT GAA ATC CTG GCG GTG CTC GGG Ala Arg Glu Arg Arg Asp Met Gln Arg Glu Ile Leu Ala Val Leu Gly 40 45 50	248
CTA CCG GGA CGG CCC CGA CCC CGT GCA CAA CCC GCG GCT GCC CGG CAG Leu Pro Gly Arg Pro Arg Pro Arg Ala Gln Pro Ala Ala Ala Arg Gln 55 60 65	296
CCA GCG TCC GCG CCC CTC TTC ATG TTG GAC CTA TAC CAC GCC ATG ACC Pro Ala Ser Ala Pro Leu Phe Met Leu Asp Leu Tyr His Ala Met Thr 70 75 80	344
GAT GAC GAC GAC GGC GGG CCA CCA CAG GCT CAC TTA GGC CGT GCC GAC Asp Asp Asp Asp Gly Gly Pro Pro Gln Ala His Leu Gly Arg Ala Asp 85 90 95 100	392
CTG GTC ATG AGC TTC GTC AAC ATG GTG GAA CGC GAC CGT ACC CTG GGC Leu Val Met Ser Phe Val Asn Met Val Glu Arg Asp Arg Thr Leu Gly 105 110 115	440
TAC CAG GAG CCA CAC TGG AAG GAA TTC CAC TTT GAC CTA ACC CAG ATC Tyr Gln Glu Pro His Trp Lys Glu Phe His Phe Asp Leu Thr Gln Ile 120 125 130	488
CCT GCT GGG GAG GCT GTC ACA GCT GCT GAG TTC CGG ATC TAC AAA GAA Pro Ala Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Glu 135 140 145	536
CCC AGC ACC CAC CCG CTC AAC ACA ACC CTC CAC ATC AGC ATG TTC GAA Pro Ser Thr His Pro Leu Asn Thr Thr Leu His Ile Ser Met Phe Glu 150 155 160	584
GTG GTC CAA GAG CAC TCC AAC AGG GAG TCT GAC TTG TTC TTT TTG GAT Val Val Gln Glu His Ser Asn Arg Glu Ser Asp Leu Phe Phe Leu Asp 165 170 175 180	632
CTT CAG ACG CTC CGA TCT GGG GAC GAG GGC TGG CTG GTG CTG GAC ATC Leu Gln Thr Leu Arg Ser Gly Asp Glu Gly Trp Leu Val Leu Asp Ile 185 190 195	680
ACA GCA GCC AGT GAC CGA TGG CTG CTG AAC CAT CAC AAG GAC CTG GGA Thr Ala Ala Ser Asp Arg Trp Leu Leu Asn His His Lys Asp Leu Gly 200 205 210	728
CTC CGC CTC TAT GTG GAA ACC GCG GAT GGG CAC AGC ATG GAT CCT GGC Leu Arg Leu Tyr Val Glu Thr Ala Asp Gly His Ser Met Asp Pro Gly 215 220 225	776
CTG GCT GGT CTG CTT GGA CGA CAA GCA CCA CGC TCC AGA CAG CCT TTC Leu Ala Gly Leu Leu Gly Arg Gln Ala Pro Arg Ser Arg Gln Pro Phe 230 235 240	824

469260 "934650

ATG GTA ACC TTC TTC AGG GCC AGC CAG AGT CCT GTG CGG GCC CCT CGG Met Val Thr Phe Phe Arg Ala Ser Gln Ser Pro Val Arg Ala Pro Arg 245 250 255 260	872
GCA GCG AGA CCA CTG AAG AGG AGG CAG CCA AAG AAA ACG AAC GAG CTT Ala Ala Arg Pro Leu Lys Arg Arg Gln Pro Lys Lys Thr Asn Glu Leu 265 270 275	920
CCG CAC CCC AAC AAA CTC CCA GGG ATC TTT GAT GAT GGC CAC GGT TCC Pro His Pro Asn Lys Leu Pro Gly Ile Phe Asp Asp Gly His Gly Ser 280 285 290	968
CGC GGC AGA GAG GTT TGC CGC AGG CAT GAG CTC TAC GTC AGC TTC CGT Arg Gly Arg Glu Val Cys Arg Arg His Glu Leu Tyr Val Ser Phe Arg 295 300 305	1016
GAC CTT GGC TGG CTG GAC TGG GTC ATC GCC CCC CAG GGC TAC TCT GCC Asp Leu Gly Trp Leu Asp Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala 310 315 320	1064
TAT TAC TGT GAG GGG GAG TGT GCT TTC CCA CTG GAC TCC TGT ATG AAC Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asp Ser Cys Met Asn 325 330 335 340	1112
GCC ACC AAC CAT GCC ATC TTG CAG TCT CTG GTG CAC CTG ATG AAG CCA Ala Thr Asn His Ala Ile Leu Gln Ser Leu Val His Leu Met Lys Pro 345 350 355	1160
GAT GTT GTC CCC AAG GCA TGC TGT GCA CCC ACC AAA CTG AGT GCC ACC Asp Val Val Pro Lys Ala Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr 360 365 370	1208
TCT GTG CTG TAC TAT GAC AGC AGC AAC AAT GTC ATC CTG CGT AAA CAC Ser Val Leu Tyr Tyr Asp Ser Ser Asn Asn Val Ile Leu Arg Lys His 375 380 385	1256
CGT AAC ATG GTG GTC AAG GCC TGT GGC TGC CAC TGAGGCCCCG CCCAGCATCC Arg Asn Met Val Val Lys Ala Cys Gly Cys His 390 395	1309
TGCTTCTACT ACCTTACCAT CTGGCCGGGC CCCTCTCCAG AGGCAGAAAC CCTTCTATGT	1369
TATCATAGCT CAGACAGGGG CAATGGGAGG CCCTTCACTT CCCCTGGCCA CTTCTGCTA	1429
AAATTCTGGT CTTTCCCAGT TCCTCTGTCC TTCATGGGGT TTCGGGGCTA TCACCCCGCC	1489
CTCTCCATCC TCCTACCCCA AGCATAGACT GAATGCACAC AGCATCCCAG AGCTATGCTA	1549
ACTGAGAGGT CTGGGGTCAG CACTGAAGGC CCACATGAGG AAGACTGATC CTTGGCCATC	1609
CTCAGCCCAC AATGGCAAAT TCTGGATGGT CTAAGAAGGC CGTGGAATTC TAAACTAGAT	1669

GATCTGGGCT CTCTGCACCA TTCATTGTGG CAGTTGGGAC ATTTTtagGT ATAACAGACA 1729
 CATACTTA GATCAATGCA TCGCTGTACT CCTTGAAATC AGAGCTAGCT TGTTAGAAAA 1789
 AGAATCAGAG CCAGGTATAG CGGTGCATGT CATTAAATCCC AGCGCTAAAG AGACAGAGAC 1849
 AGGAGAATCT CTGTGAGTTC AAGGCCACAT AGAAAGAGCC TGTCTCGGGA GCAGGAAAAA 1909
 AAAAAAAAAAC GGAATTC 1926

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 399 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- (D) OTHER INFORMATION: /product= "mOP2-PP"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Met Ala Met Arg Pro Gly Pro Leu Trp Leu Leu Gly Leu Ala Leu Cys
 1 5 10 15
 Ala Leu Gly Gly Gly His Gly Pro Arg Pro Pro His Thr Cys Pro Gln
 20 25 30
 Arg Arg Leu Gly Ala Arg Glu Arg Arg Asp Met Gln Arg Glu Ile Leu Ala
 35 40 45
 Val Leu Gly Leu Pro Gly Arg Pro Arg Pro Arg Ala Gln Pro Ala Ala
 50 55 60 65
 Ala Arg Gln Pro Ala Ser Ala Pro Leu Phe Met Leu Asp Leu Tyr His Ala
 70 75 80
 Met Thr Asp Asp Asp Gly Gly Pro Pro Gln Ala His Leu Gly Arg
 85 90 95
 Ala Asp Leu Val Met Ser Phe Val Asn Met Val Glu Arg Asp Arg Thr
 100 105 110
 Leu Gly Tyr Gln Glu Pro His Trp Lys Glu Phe His Phe Asp Leu Thr
 115 120 125 130
 Gln Ile Pro Ala Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile Tyr
 135 140 145

263250"9342680

Lys Glu Pro Ser Thr His Pro Leu Asn Thr Thr Leu His Ile Ser Met
 150 155 160
 Phe Glu Val Val Gln Glu His Ser Asn Arg Glu Ser Asp Leu Phe Phe
 165 170 175
 Leu Asp Leu Gln Thr Leu Arg Ser Gly Asp Glu Gly Trp Leu Val Leu
 180 185 190
 Asp Ile Thr Ala Ala Ser Asp Arg Trp Leu Leu Asn His His Lys Asp
 195 200 205 210
 Leu Gly Leu Arg Leu Tyr Val Glu Thr Ala Asp Gly His Ser Met Asp
 215 220 225
 Pro Gly Leu Ala Gly Leu Leu Gly Arg Gln Ala Pro Arg Ser Arg Gln
 230 235 240
 Pro Phe Met Val Thr Phe Phe Arg Ala Ser Gln Ser Pro Val Arg Ala
 245 250 255
 Pro Arg Ala Ala Arg Pro Leu Lys Arg Arg Gln Pro Lys Lys Thr Asn
 260 265 270
 Glu Leu Pro His Pro Asn Lys Leu Pro Gly Ile Phe Asp Asp Gly His
 275 280 285 290
 Gly Ser Arg Gly Arg Glu Val Cys Arg Arg His Glu Leu Tyr Val Ser
 295 300 305
 Phe Arg Asp Leu Gly Trp Leu Asp Trp Val Ile Ala Pro Gln Gly Tyr
 310 315 320
 Ser Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asp Ser Cys
 325 330 335
 Met Asn Ala Thr Asn His Ala Ile Leu Gln Ser Leu Val His Leu Met
 340 345 350
 Lys Pro Asp Val Val Pro Lys Ala Cys Cys Ala Pro Thr Lys Leu Ser
 355 360 365 370
 Ala Thr Ser Val Leu Tyr Tyr Asp Ser Ser Asn Asn Val Ile Leu Arg
 375 380 385
 Lys His Arg Asn Met Val Val Lys Ala Cys Gly Cys His
 390 395

(2) INFORMATION FOR SEQ ID NO:24:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1368 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 1..1368
(D) OTHER INFORMATION:/STANDARD NAME="60A"

(x) PUBLICATION INFORMATION:

(A) AUTHORS: WHARTON, KRISTI A.; THOMSEN, GERALD H.;
GELBERT, WILLIAM M.
(B) TITLE: DROSOPHILA 60A GENE...
(C) JOURNAL: PROC. NAT'L ACAD. SCI. USA
(D) VOLUME: 88
(E) RELEVANT RESIDUES IN SEQ ID NO:3: FROM 1 TO 1368
(F) PAGES: 9214-9218
(G) DATE: OCT - 1991

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

ATG TCG GGA CTG CGA AAC ACC TCG GAG GCC GTT GCA GTG CTC GCC TCC	48
Met Ser Gly Leu Arg Asn Thr Ser Glu Ala Val Ala Val Leu Ala Ser	
1 5 10 15	
CTG GGA CTC GGA ATG GTT CTG CTC ATG TTC GTG GCG ACC ACG CCG CCG	96
Leu Gly Leu Gly Met Val Leu Leu Met Phe Val Ala Thr Thr Pro Pro	
20 25 30	
GCC GTT GAG GCC ACC CAG TCG GGG ATT TAC ATA GAC AAC GGC AAG GAC	144
Ala Val Glu Ala Thr Gln Ser Gly Ile Tyr Ile Asp Asn Gly Lys Asp	
35 40 45	
CAG ACG ATC ATG CAC AGA GTG CTG AGC GAG GAC GAC AAG CTG GAC GTC	192
Gln Thr Ile Met His Arg Val Leu Ser Glu Asp Asp Lys Leu Asp Val	
50 55 60	
TCG TAC GAG ATC CTC GAG TTC CTG GGC ATC GCC GAA CGG CCG ACG CAC	240
Ser Tyr Glu Ile Leu Glu Phe Leu Gly Ile Ala Glu Arg Pro Thr His	
65 70 75 80	
CTG AGC AGC CAC CAG TTG TCG CTG AGG AAG TCG GCT CCC AAG TTC CTG	288
Leu Ser Ser His Gln Leu Ser Leu Arg Lys Ser Ala Pro Lys Phe Leu	
85 90 95	
CTG GAC GTC TAC CAC CGC ATC ACG GCG GAG GAG GGT CTC AGC GAT CAG	336
Leu Asp Val Tyr His Arg Ile Thr Ala Glu Glu Gly Leu Ser Asp Gln	
100 105 110	

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GAT Asp	GAG Glu	GAC Asp 115	GAC Asp	GAC Asp	TAC Tyr	GAA Glu 120	CGC Arg	GGC Gly	CAT His	CGG Arg	TCC Ser	AGG Arg 125	AGG Arg	AGC Ser	GCC Ala	384
GAC Asp 130	CTC Leu	GAG Glu	GAG Glu	GAT Asp	GAG Glu	GGC Gly 135	GAG Glu	CAG Gln	CAG Gln	AAG Lys	AAC Asn 140	TTC Phe	ATC Ile	ACC Thr	GAC Asp	432
CTG Leu 145	GAC Asp	AAG Lys	CGG Arg	GCC Ala	ATC Ile 150	GAC Asp	GAG Glu	AGC Ser	GAC Asp	ATC Ile 155	ATC Ile	ATG Met	ACC Thr	TTC Phe	CTG Leu 160	480
AAC Asn	AAG Lys	CGC Arg	CAC His 165	CAC His	AAT Asn	GTG Val	GAC Asp	GAA Glu	CTG Leu 170	CGT Arg	CAC His	GAG Glu	CAC His	GGC Gly 175	CGT Arg	528
CGC Arg	CTG Leu	TGG Trp	TTC Phe 180	GAC Asp	GTC Val	TCC Ser	AAC Asn	GTG Val 185	CCC Pro	AAC Asn	GAC Asp	AAC Asn	TAC Tyr 190	CTG Leu	GTG Val	576
ATG Met	GCC Ala	GAG Glu 195	CTG Leu	CGC Arg	ATC Ile	TAT Tyr	CAG Gln 200	AAC Asn	GCC Ala	AAC Asn	GAG Glu	GGC Gly 205	AAG Lys	TGG Trp	CTG Leu	624
ACC Thr 210	GCC Ala	AAC Asn	AGG Arg	GAG Glu	TTC Phe 215	ACC Thr	ATC Ile	ACG Thr	GTA Val	TAC Tyr	GCC Ala 220	ATT Ile	GGC Gly	ACC Thr	GGC Gly	672
ACG Thr 225	CTG Leu	GGC Gly	CAG Gln	CAC His	ACC Thr 230	ATG Met	GAG Glu	CCG Pro	CTG Leu	TCC Ser 235	TCG Ser	GTG Val	AAC Asn	ACC Thr	ACC Thr 240	720
GGG Gly	GAC Asp	TAC Tyr	GTG Val 245	GGC Gly	TGG Trp	TTG Leu	GAG Glu	CTC Leu	AAC Asn 250	GTG Val	ACC Thr	GAG Glu	GGC Gly	CTG Leu 255	CAC His	768
GAG Glu	TGG Trp	CTG Leu	GTC Val 260	AAG Lys	TCG Ser	AAG Lys	GAC Asp	AAT Asn 265	CAT His	GGC Gly	ATC Ile	TAC Tyr	ATT Ile 270	GGA Gly	GCA Ala	816
CAC His	GCT Ala	GTC Val 275	AAC Asn	CGA Arg	CCC Pro	GAC Asp	CGC Arg 280	GAG Glu	GTG Val	AAG Lys	CTG Leu	GAC Asp 285	GAC Asp	ATT Ile	GGA Gly	864
CTG Leu 290	ATC Ile	CAC His	CGC Arg	AAG Lys	GTG Val 295	GAC Asp	GAC Asp	GAG Glu	TTC Phe	CAG Gln	CCC Pro 300	TTC Phe	ATG Met	ATC Ile	GGC Gly	912
TTC Phe 305	TTC Phe	CGC Arg	GGA Gly	CCG Pro	GAG Glu 310	CTG Leu	ATC Ile	AAG Lys	GCG Ala	ACG Thr 315	GCC Ala	CAC His	AGC Ser	AGC Ser	CAC His 320	960

CAC AGG AGC AAG CGA AGC GCC AGC CAT CCA CGC AAG CGC AAG AAG TCG	1008
His Arg Ser Lys Arg Ser Ala Ser His Pro Arg Lys Arg Lys Lys Ser	
325 330 335	
GTG TCG CCC AAC AAC GTG CCG CTG CTG GAA CCG ATG GAG AGC ACG CGC	1056
Val Ser Pro Asn Asn Val Pro Leu Leu Glu Pro Met Glu Ser Thr Arg	
340 345 350	
AGC TGC CAG ATG CAG ACC CTG TAC ATA GAC TTC AAG GAT CTG GGC TGG	1104
Ser Cys Gln Met Gln Thr Leu Tyr Ile Asp Phe Lys Asp Leu Gly Trp	
355 360 365	
CAT GAC TGG ATC ATC GCA CCA GAG GGC TAT GGC GCC TTC TAC TGC AGC	1152
His Asp Trp Ile Ile Ala Pro Glu Gly Tyr Gly Ala Phe Tyr Cys Ser	
370 375 380	
GGC GAG TGC AAT TTC CCG CTC AAT GCG CAC ATG AAC GCC ACG AAC CAT	1200
Gly Glu Cys Asn Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His	
385 390 395 400	
CGC ATC GTC CAG ACC CTG GTC CAC CTG CTG GAG CCC AAG AAG GTG CCC	1248
Ala Ile Val Gln Thr Leu Val His Leu Leu Glu Pro Lys Lys Val Pro	
405 410 415	
AAG CCC TGC TGC GCT CCG ACC AGG CTG GGA GCA CTA CCC GTT CTG TAC	1296
Lys Pro Cys Cys Ala Pro Thr Arg Leu Gly Ala Leu Pro Val Leu Tyr	
420 425 430	
CAC CTG AAC GAC GAG AAT GTG AAC CTG AAA AAG TAT AGA AAC ATG ATT	1344
His Leu Asn Asp Glu Asn Val Asn Leu Lys Lys Tyr Arg Asn Met Ile	
435 440 445	
GTG AAA TCC TGC GGG TGC CAT TGA	1368
Val Lys Ser Cys Gly Cys His	
450 455	

(2) INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 455 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Met	Ser	Gly	Leu	Arg	Asn	Thr	Ser	Glu	Ala	Val	Ala	Val	Leu	Ala	Ser
1				5					10					15	

Leu	Gly	Leu	Gly	Met	Val	Leu	Leu	Met	Phe	Val	Ala	Thr	Thr	Pro	Pro
		20						25					30		
Ala	Val	Glu	Ala	Thr	Gln	Ser	Gly	Ile	Tyr	Ile	Asp	Asn	Gly	Lys	Asp
		35					40					45			
Gln	Thr	Ile	Met	His	Arg	Val	Leu	Ser	Glu	Asp	Asp	Lys	Leu	Asp	Val
	50					55					60				
Ser	Tyr	Glu	Ile	Leu	Glu	Phe	Leu	Gly	Ile	Ala	Glu	Arg	Pro	Thr	His
65					70					75					80
Leu	Ser	Ser	His	Gln	Leu	Ser	Leu	Arg	Lys	Ser	Ala	Pro	Lys	Phe	Leu
				85					90					95	
Leu	Asp	Val	Tyr	His	Arg	Ile	Thr	Ala	Glu	Glu	Gly	Leu	Ser	Asp	Gln
			100					105					110		
Asp	Glu	Asp	Asp	Asp	Tyr	Glu	Arg	Gly	His	Arg	Ser	Arg	Arg	Ser	Ala
		115					120					125			
Asp	Leu	Glu	Glu	Asp	Glu	Gly	Glu	Gln	Gln	Lys	Asn	Phe	Ile	Thr	Asp
	130					135					140				
Leu	Asp	Lys	Arg	Ala	Ile	Asp	Glu	Ser	Asp	Ile	Ile	Met	Thr	Phe	Leu
145					150					155					160
Asn	Lys	Arg	His	His	Asn	Val	Asp	Glu	Leu	Arg	His	Glu	His	Gly	Arg
				165					170					175	
Arg	Leu	Trp	Phe	Asp	Val	Ser	Asn	Val	Pro	Asn	Asp	Asn	Tyr	Leu	Val
			180					185					190		
Met	Ala	Glu	Leu	Arg	Ile	Tyr	Gln	Asn	Ala	Asn	Glu	Gly	Lys	Trp	Leu
		195					200					205			
Thr	Ala	Asn	Arg	Glu	Phe	Thr	Ile	Thr	Val	Tyr	Ala	Ile	Gly	Thr	Gly
	210					215					220				
Thr	Leu	Gly	Gln	His	Thr	Met	Glu	Pro	Leu	Ser	Ser	Val	Asn	Thr	Thr
225					230					235					240
Gly	Asp	Tyr	Val	Gly	Trp	Leu	Glu	Leu	Asn	Val	Thr	Glu	Gly	Leu	His
				245					250					255	
Glu	Trp	Leu	Val	Lys	Ser	Lys	Asp	Asn	His	Gly	Ile	Tyr	Ile	Gly	Ala
			260					265					270		
His	Ala	Val	Asn	Arg	Pro	Asp	Arg	Glu	Val	Lys	Leu	Asp	Asp	Ile	Gly
		275					280					285			
Leu	Ile	His	Arg	Lys	Val	Asp	Asp	Glu	Phe	Gln	Pro	Phe	Met	Ile	Gly
	290					295					300				

Phe Phe Arg Gly Pro Glu Leu Ile Lys Ala Thr Ala His Ser Ser His
 305 310 315 320

His Arg Ser Lys Arg Ser Ala Ser His Pro Arg Lys Arg Lys Lys Ser
 325 330 335

Val Ser Pro Asn Asn Val Pro Leu Leu Glu Pro Met Glu Ser Thr Arg
 340 345 350

Ser Cys Gln Met Gln Thr Leu Tyr Ile Asp Phe Lys Asp Leu Gly Trp
 355 360 365

His Asp Trp Ile Ile Ala Pro Glu Gly Tyr Gly Ala Phe Tyr Cys Ser
 370 375 380

Gly Glu Cys Asn Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His
 385 390 395 400

Ala Ile Val Gln Thr Leu Val His Leu Leu Glu Pro Lys Lys Val Pro
 405 410 415

Lys Pro Cys Cys Ala Pro Thr Arg Leu Gly Ala Leu Pro Val Leu Tyr
 420 425 430

His Leu Asn Asp Glu Asn Val Asn Leu Lys Lys Tyr Arg Asn Met Ile
 435 440 445

Val Lys Ser Cys Gly Cys His
 450 455

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens

(ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..102
- (D) OTHER INFORMATION: /note="BMP3"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 104 amino acids
- (B) TYPE: amino acid

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- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii)MOLECULE TYPE: protein

(ix)FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..104
- (D) OTHER INFORMATION: /note="BMP3"

(xi)SEQUENCE DESCRIPTION: SEQ ID NO:26:

Cys Ala Arg Arg Tyr Leu Lys Val Asp Phe Ala Asp Ile Gly Trp Ser
1 5 10 15
Glu Trp Ile Ile Ser Pro Lys Ser Phe Asp Ala Tyr Try Cys Ser Gly
20 25 30
Ala Cys Gln Phe Pro Met Pro Lys Ser Leu Lys Pro Ser Asn His Ala
35 40 45
Thr Ile Gln Ser Ile Val Ala Arg Ala Val Gly Val Val Pro Gly Ile
50 55 60
Pro Glu Pro Cys Cys Val Pro Glu Lys Met Ser Ser Leu Ser Ile Leu
65 70 75 80
Phe Phe Asp Glu Asn Lys Asn Val Val Leu Lys Val Tyr Pro Asn Met
85 90 95
Thr Val Glu Ser Cys Ala Cys Arg
100

(2) INFORMATION FOR SEQ ID NO:27:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 102 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: HOMO SAPIENS

(ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..102
- (D) OTHER INFORMATION: /note= "BMP5"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg Asp Leu Gly Trp Gln
 1 5 10 15
 Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala Phe Tyr Cys Asp Gly
 20 25 30
 Glu Cys Ser Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His Ala
 35 40 45
 Ile Val Gln Thr Leu Val His Leu Met Phe Pro Asp His Val Pro Lys
 50 55 60
 Pro Cys Cys Ala Pro Thr Lys Leu Asn Ala Ile Ser Val Leu Tyr Phe
 65 70 75 80
 Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr Arg Asn Met Val Val
 85 90 95
 Arg Ser Cys Gly Cys His
 100

(2) INFORMATION FOR SEQ ID NO:28:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 102 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: HOMO SAPIENS
- (ix) FEATURE:
 - (A) NAME/KEY: Protein
 - (B) LOCATION: 1..102
 - (D) OTHER INFORMATION: /note= "BMP6"
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Cys Arg Lys His Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Gln
 1 5 10 15
 Asp Trp Ile Ile Ala Pro Lys Gly Tyr Ala Ala Asn Tyr Cys Asp Gly
 20 25 30
 Glu Cys Ser Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His Ala
 35 40 45

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Ile Val Gln Thr Leu Val His Leu Met Asn Pro Glu Tyr Val Pro Lys
50 55 60

Pro Cys Cys Ala Pro Thr Lys Leu Asn Ala Ile Ser Val Leu Tyr Phe
65 70 75 80

Asp Asp Asn Ser Asn Val Ile Leu Lys Lys Tyr Arg Trp Met Val Val
85 90 95

Arg Ala Cys Gly Cys His
100

(2) INFORMATION FOR SEQ ID NO:29:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 102 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

- (ix) FEATURE:
 - (A) NAME/KEY: Protein
 - (B) LOCATION: 1..102
 - (D) OTHER INFORMATION: /label= OPX
/note= "WHEREIN XAA AT EACH POS'N IS INDEPENDENTLY
SELECTED FROM THE RESIDUES OCCURRING AT THE
CORRESPONDING POS'N IN THE C-TERMINAL SEQUENCE OF MOUSE
OR HUMAN OP1 OR OP2 (SEE SEQ. ID NOS. 5,6,7 and 8 or
16,18,20 and 22.)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Cys Xaa Xaa His Glu Leu Tyr Val Xaa Phe Xaa Asp Leu Gly Trp Xaa
1 5 10 15

Asp Trp Xaa Ile Ala Pro Xaa Gly Tyr Xaa Ala Tyr Tyr Cys Glu Gly
20 25 30

Glu Cys Xaa Phe Pro Leu Xaa Ser Xaa Met Asn Ala Thr Asn His Ala
35 40 45

Ile Xaa Gln Xaa Leu Val His Xaa Xaa Xaa Pro Xaa Xaa Val Pro Lys
50 55 60

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Xaa Cys Cys Ala Pro Thr Xaa Leu Xaa Ala Xaa Ser Val Leu Tyr Xaa
65 70 75 80
Asp Xaa Ser Xaa Asn Val Xaa Leu Xaa Lys Xaa Arg Asn Met Val Val
85 90 95
Xaa Ala Cys Gly Cys His
100

(2) INFORMATION FOR SEQ ID NO:30:

(i)SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 97 amino acids
- (B) TYPE: amino acids
- (C) TOPOLOGY: linear

(ii)MOLECULE TYPE: protein

(ix)FEATURE:

- (A) NAME: Generic Sequence 5
- (D) OTHER INFORMATION: wherein each Xaa is independently selected from a group of one or more specified amino acids as defined in the specification.

(xi)SEQUENCE DESCRIPTION: SEQ ID NO:30:

Leu Xaa Xaa Xaa Phe
1 5
Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa
10
Xaa Xaa Pro Xaa Xaa Xaa Xaa Ala
15 20
Xaa Tyr Cys Xaa Gly Xaa Cys Xaa
25 30
Xaa Pro Xaa Xaa Xaa Xaa Xaa
35
Xaa Xaa Xaa Asn His Ala Xaa Xaa
40 45
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
50
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
55 60
Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa
65
Xaa Xaa Xaa Leu Xaa Xaa Xaa
70 75
Xaa Xaa Xaa Xaa Val Xaa Leu Xaa
80
Xaa Xaa Xaa Xaa Met Xaa Val Xaa
85 90
Xaa Cys Xaa Cys Xaa
95

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(2) INFORMATION FOR SEQ ID NO:31:

(i)SEQUENCE CHARACTERISTICS:

(A) LENGTH: 102 amino acids

(B) TYPE: amino acids

(C) TOPOLOGY: linear

(ii)MOLECULE TYPE: protein

(ix)FEATURE:

(A) NAME: Generic Sequence 6

(D) OTHER INFORMATION: wherein each Xaa is independently selected from a group of one or more specified amino acids as defined in the specification.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Cys Xaa Xaa Xaa Xaa Leu Xaa Xaa Xaa Phe

1 5 10

Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa

15

Xaa Xaa Pro Xaa Xaa Xaa Xaa Ala

20 25

Xaa Tyr Cys Xaa Gly Xaa Cys Xaa

30 35

Xaa Pro Xaa Xaa Xaa Xaa Xaa

40

Xaa Xaa Xaa Asn His Ala Xaa Xaa

45 50

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa

55

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys

60 65

Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa

70

Xaa Xaa Xaa Leu Xaa Xaa Xaa

75 80

Xaa Xaa Xaa Xaa Val Xaa Leu Xaa

85

Xaa Xaa Xaa Xaa Met Xaa Val Xaa

90

95

Xaa Cys Xaa Cys Xaa

100

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1238 base pairs, 372 amino acids
- (B) TYPE: nucleic acid, amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) ORIGINAL SOURCE:

- (A) ORGANISM: human
- (F) TISSUE TYPE: BRAIN

(iv) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION:
- (D) OTHER INFORMATION:
 - /product= "GDF-1"
 - /note= "GDF-1 CDNA"

(x) PUBLICATION INFORMATION:

- (A) AUTHORS: Lee, Se-Jin
- (B) TITLE: Expression of Growth/Differentiation Factor 1
- (C) JOURNAL: Proc. Nat'l Acad. Sci.
- (D) VOLUME: 88
- (E) RELEVANT RESIDUES: 1-1238
- (F) PAGES: 4250-4254
- (G) DATE: May-1991

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

GGGACACCG GCCCGCCCT CAGCCCACTG GTCCCGGGCC GCCGCGGACC CTGCGCACTC 60

TCTGGTCATC GCCTGGGAGG AAG ATG CCA CCG CCG CAG CAA GGT CCC TGC GGC 113
Met Pro Pro Pro Gln Gln Gly Pro Cys Gly
1 5 10

CAC CAC CTC CTC CTC CTC CTG GCC CTG CTG CTG CCC TCG CTG CCC 158
His His Leu Leu Leu Leu Leu Ala Leu Leu Leu Pro Ser Leu Pro
15 20 25

CTG	ACC	CGC	GCC	CCC	GTG	CCC	CCA	GGC	CCA	GCC	GCC	GCC	CTG	CTC	203
Leu	Thr	Arg	Ala	Pro	Val	Pro	Pro	Gly	Pro	Ala	Ala	Ala	Leu	Leu	
				30					35					40	
CAG	GCT	CTA	GGA	CTG	CGC	GAT	GAG	CCC	CAG	GGT	GCC	CCC	AGG	CTC	248
Gln	Ala	Leu	Gly	Leu	Arg	Asp	Glu	Pro	Gln	Gly	Ala	Pro	Arg	Leu	
				45					50					55	
CGG	CCG	GTT	CCC	CCG	GTC	ATG	TGG	CGC	CTG	TTT	CGA	CGC	CGG	GAC	293
Arg	Pro	Val	Pro	Pro	Val	Met	Trp	Arg	Leu	Phe	Arg	Arg	Arg	Asp	
				60					65					70	
CCC	CAG	GAG	ACC	AGG	TCT	GGC	TCG	CGG	CGG	ACG	TCC	CCA	GGG	GTC	338
Pro	Gln	Glu	Thr	Arg	Ser	Gly	Ser	Arg	Arg	Thr	Ser	Pro	Gly	Val	
				75					80					85	
ACC	CTG	CAA	CCG	TGC	CAC	GTG	GAG	GAG	CTG	GGG	GTC	GCC	GGA	AAC	383
Thr	Leu	Gln	Pro	Cyc	His	Val	Glu	Glu	Leu	Gly	Val	Ala	Gly	Asn	
				90					95					100	
ATC	GTG	CGC	CAC	ATC	CCG	GAC	CGC	GGT	GCG	CCC	ACC	CGG	GCC	TCG	428
Ile	Val	Arg	His	Ile	Pro	Asp	Arg	Gly	Ala	Pro	Thr	Arg	Ala	Ser	
				105					110					115	
GAG	CCT	GTC	TCG	GCC	GCG	GGG	CAT	TGC	CCT	GAG	TGG	ACA	GTC	GTC	473
Glu	Pro	Val	Ser	Ala	Ala	Gly	His	Cys	Pro	Glu	Trp	Thr	Val	Val	
				120					125					130	
TTC	GAC	CTG	TCG	GCT	GTG	GAA	CCC	GCT	GAG	CGC	CCG	AGC	CGG	GCC	518
Phe	Asp	Leu	Ser	Ala	Val	Glu	Pro	Ala	Glu	Arg	Pro	Ser	Arg	Ala	
				135					140					145	
CGC	CTG	GAG	CTG	CGT	TTC	GCG	GCG	GCG	GCG	GCG	GCA	GCC	CCG	GAG	563
Arg	Leu	Glu	Leu	Arg	Phe	Ala	Ala	Ala	Ala	Ala	Ala	Ala	Pro	Glu	
				150					155					160	
GGC	GGC	TGG	GAG	CTG	AGC	GTG	GCG	CAA	GCG	GGC	CAG	GGC	GCG	GGC	608
Gly	Gly	Trp	Glu	Leu	Ser	Val	Ala	Gln	Ala	Gly	Gln	Gly	Ala	Gly	
				165					170					175	
GCG	GAC	CCC	GGG	CCG	GTG	CTG	CTC	CGC	CAG	TTG	GTG	CCC	GCC	CTG	653
Ala	Asp	Pro	Gly	Pro	Val	Leu	Leu	Arg	Gln	Leu	Val	Pro	Ala	Leu	
				180					185					190	
GGG	CCG	CCA	GTG	CGC	GCG	GAG	CTG	CTG	GGC	GCC	GCT	TGG	GCT	CGC	698
Gly	Pro	Pro	Val	Arg	Ala	Glu	Leu	Leu	Gly	Ala	Ala	Trp	Ala	Arg	
				195					200					205	
AAC	GCC	TCA	TGG	CCG	CGC	AGC	CTC	CGC	CTG	GCG	CTG	GCG	CTA	CGC	743
Asn	Ala	Ser	Trp	Pro	Arg	Ser	Leu	Arg	Leu	Ala	Leu	Ala	Leu	Arg	
				210					215					220	

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CCC CGG GCC CCT GCC GCC TGC GCG CGC CTG GCC GAG GCC TCG CTG	788
Pro Arg Ala Pro Ala Ala Cys Ala Arg Leu Ala Glu Ala Ser Leu	
225 230 235	
CTG CTG GTG ACC CTC GAC CCG CGC CTG TGC CAC CCC CTG GCC CGG	833
Leu Leu Val Thr Leu Asp Pro Arg Leu Cys His Pro Leu Ala Arg	
240 245 250	
CCG CGG CGC GAC GCC GAA CCC GTG TTG GGC GGC GGC CCC GGG GGC	878
Pro Arg Arg Asp Ala Glu Pro Val Leu Gly Gly Gly Pro Gly Gly	
255 260 265	
GCT TGT CGC GCG CGG CGG CTG TAC GTG AGC TTC CGC CAG GTG GGC	923
Ala Cys Arg Ala Arg Arg Leu Tyr Val Ser Phe Arg Glu Val Gly	
270 275 280	
TGG CAC CGC TGG GTC ATC GCG CCG CGC CCC TTC CTG GCC AAC TAC	968
Trp His Arg Trp Val Ile Arg Pro Arg Gly Phe Leu Ala Asn Tyr	
285 290 295	
TGC CAG GGT CAG TGC GCG CTG CCC GTC GCG CTG TCG GGG TCC GGG	1013
Cys Gln Gly Gln Cys Ala Leu Pro Val Ala Leu Ser Gly Ser Gly	
300 305 310	
GGG CCG CCG GCG CTC AAC CAC GCT GTG CTG CGC GCG CTC ATG CAC	1058
Gly Pro Pro Ala Leu Asn His Ala Val Leu Arg Ala Leu Met His	
315 320 325	
GCG GCC GCC CCG GGA GCC GCC GAC CTG CCC TGC TGC GTG CCC GCG	1103
Ala Ala Ala Pro Gly Ala Ala Asp Leu Pro Cys Cys Val Pro Ala	
330 335 340	
CGC CTG TCG CCC ATC TCC GTG CTC TTC TTT GAC AAC AGC GAC AAC	1148
Arg Leu Ser Pro Ile Ser Val Leu Phe Phe Asp Asn Ser Asp Asn	
345 350 355	
GTG GTG CTG CGG CAG TAT GAG GAC ATG GTG GTG GAC GAG TGC GGC	1193
Val Val Leu Arg Gln Tyr Glu Asp Met Val Val Asp Glu Cys Gly	
360 365 370	
TGC CGC TAACCCGGGG CGGGCAGGGA CCCGGGCCCA ACAATAAATG CCGCGTGG	1238
Cys Arg	
372	

(34) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 372 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

														Met	Pro	Pro	Pro	Gln	Gln	Gly	Pro	Cys	Gly
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His	His	Leu	Leu	Leu	Leu	Leu	Ala	Leu	Leu	Leu	Pro	Ser	Leu	Pro									
				15					20					25									
Leu	Thr	Arg	Ala	Pro	Val	Pro	Pro	Gly	Pro	Ala	Ala	Ala	Leu	Leu									
				30					35					40									
Gln	Ala	Leu	Gly	Leu	Arg	Asp	Glu	Pro	Gln	Gly	Ala	Pro	Arg	Leu									
				45					50					55									
Arg	Pro	Val	Pro	Pro	Val	Met	Trp	Arg	Leu	Phe	Arg	Arg	Arg	Asp									
				60					65					70									
Pro	Gln	Glu	Thr	Arg	Ser	Gly	Ser	Arg	Arg	Thr	Ser	Pro	Gly	Val									
				75					80					85									
Thr	Leu	Gln	Pro	Cyc	His	Val	Glu	Glu	Leu	Gly	Val	Ala	Gly	Asn									
				90					95					100									
Ile	Val	Arg	His	Ile	Pro	Asp	Arg	Gly	Ala	Pro	Thr	Arg	Ala	Ser									
				105					110					115									
Glu	Pro	Val	Ser	Ala	Ala	Gly	His	Cys	Pro	Glu	Trp	Thr	Val	Val									
				120					125					130									
Phe	Asp	Leu	Ser	Ala	Val	Glu	Pro	Ala	Glu	Arg	Pro	Ser	Arg	Ala									
				135					140					145									
Arg	Leu	Glu	Leu	Arg	Phe	Ala	Ala	Ala	Ala	Ala	Ala	Ala	Pro	Glu									
				150					155					160									

Gly	Gly	Trp	Glu	Leu	Ser	Val	Ala	Gln	Ala	Gly	Gln	Gly	Ala	Gly	165	170	175
Ala	Asp	Pro	Gly	Pro	Val	Leu	Leu	Arg	Gln	Leu	Val	Pro	Ala	Leu	180	185	190
Gly	Pro	Pro	Val	Arg	Ala	Glu	Leu	Leu	Gly	Ala	Ala	Trp	Ala	Arg	195	200	205
Asn	Ala	Ser	Trp	Pro	Arg	Ser	Leu	Arg	Leu	Ala	Leu	Ala	Leu	Arg	210	215	220
Pro	Arg	Ala	Pro	Ala	Ala	Cys	Ala	Arg	Leu	Ala	Glu	Ala	Ser	Leu	225	230	235
Leu	Leu	Val	Thr	Leu	Asp	Pro	Arg	Leu	Cys	His	Pro	Leu	Ala	Arg	240	245	250
Pro	Arg	Arg	Asp	Ala	Glu	Pro	Val	Leu	Gly	Gly	Gly	Pro	Gly	Gly	255	260	265
Ala	Cys	Arg	Ala	Arg	Arg	Leu	Tyr	Val	Ser	Phe	Arg	Glu	Val	Gly	270	275	280
Trp	His	Arg	Trp	Val	Ile	Arg	Pro	Arg	Gly	Phe	Leu	Ala	Asn	Tyr	285	290	295
Cys	Gln	Gly	Gln	Cys	Ala	Leu	Pro	Val	Ala	Leu	Ser	Gly	Ser	Gly	300	305	310
Gly	Pro	Pro	Ala	Leu	Asn	His	Ala	Val	Leu	Arg	Ala	Leu	Met	His	315	320	325
Ala	Ala	Ala	Pro	Gly	Ala	Ala	Asp	Leu	Pro	Cys	Cys	Val	Pro	Ala	330	335	340
Arg	Leu	Ser	Pro	Ile	Ser	Val	Leu	Phe	Phe	Asp	Asn	Ser	Asp	Asn	345	350	355
Val	Val	Leu	Arg	Gln	Tyr	Glu	Asp	Met	Val	Val	Asp	Glu	Cys	Gly	360	365	370
Cys	Arg														372		

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What is claimed is:

1. A method for enhancing survival of neural cells at risk of dying, the method comprising providing a morphogen to said cells at a concentration and for a time sufficient to enhance survival of said cells.
2. The method of claim 1 wherein said cells are at risk of dying due to chemical or mechanical trauma to nerve tissue comprising said cells.
3. The method of claim 2 wherein said trauma comprises a transected nerve.
4. The method of claim 2 wherein said morphogen is provided to said cells prior to said trauma.
5. The method of claim 2 wherein said trauma results in demyelination of said cells.
6. The method of claim 2 wherein said trauma results from exposure of said cells to a cellular toxin.
7. The method of claim 6 wherein said toxin comprises ethanol.
8. The method of claim 1 wherein said cells are at risk of dying due to a neuropathy.
9. The method of claim 8 wherein the etiology of said neuropathy is metabolic, infectious, toxic, autoimmune, nutritional, or ischemic.

10. The method of claim 9 wherein said neuropathy comprises Parkinson's disease, Huntington's chorea, amyotrophic lateral sclerosis, multiple sclerosis or Alzheimer's disease.
11. The method of claim 1 wherein said cells are at risk of dying due a neoplastic lesion associated with nerve tissue comprising said cells.
12. The method of claim 11 wherein said lesion results from a neoplasm comprising cells of neuronal origin.
13. The method of claim 12 wherein said neoplasm comprises a neuroblastoma or a retinoblastoma.
14. The method of claim 11 wherein said lesion results from a neoplasm comprising glial cells.
15. The method of claim 1 wherein said neural cells at risk of dying comprise part of the central nervous system.
16. The method of claim 15 wherein said cells comprise striatal basal ganglia neurons.
17. The method of claim 15 wherein said cells comprise neurons of the substantia nigra.
18. The method of claim 1 wherein said cells at risk of dying comprise part of the peripheral nervous system.

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19. The method of claim 1 wherein said morphogen stimulates cell adhesion molecule production in said cells.
20. The method of claim 19 wherein said cell adhesion molecule is a nerve cell adhesion molecule.
21. The method of claim 20 wherein nerve cell adhesion molecule is selected from the group consisting of N-CAM-120, N-CAM-140 and N-CAM-180.
22. The method of claim 1 wherein said morphogen comprises an amino acid sequence sharing at least 70% homology with one of the sequences selected from the group consisting of: OP-1, OP-2, CBMP2, Vgl(fx), Vgr(fx), DPP(fx), GDF-1(fx) and 60A(fx).
23. The method of claim 22 wherein said morphogen comprises an amino acid sequence sharing at least 80% homology with one of the sequences selected from the group consisting of: OP-1, OP-2, CBMP2, Vgl(fx), Vgr(fx), DPP(fx), GDF-1(fx), and 60A (fx).
24. The method of claim 23 wherein said morphogen comprises an amino acid sequence having greater than 60% amino acid identity with the sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1.)
25. The method of claim 24 wherein said morphogen comprises an amino acid sequence having greater than 65% amino acid identity with the sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1.)

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26. The method of claim 21 wherein said morphogen comprises an amino acid sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1), including allelic and species variants thereof.
27. A method for enhancing the survival of neural cells at risk of dying in a mammal, the method comprising the step of administering to said mammal an effective amount of an agent capable of stimulating production of an endogenous morphogen.
28. The method of claim 27 wherein said agent stimulates production of an endogenous morphogen in the tissue comprising said neural cells.
29. A method for maintaining a neural pathway in a mammal, comprising:
 - providing a morphogen to the neurons defining said pathway at a concentration and for a time sufficient to maintain said pathway.
30. The method of claim 29 wherein said morphogen is provided prior to injury to said pathway.
31. The method of claim 29 wherein said morphogen is sufficient to stimulate repair of a damaged neural pathway.
32. The method of claim 31 wherein said damaged neural pathway results from mechanical or chemical trauma to said pathway.
33. The method of claim 32 wherein said trauma comprises a severed nerve.

34. The method of claim 32 wherein said trauma comprises demyelination of the neurons defining said pathway.
35. The method of claim 32 wherein said trauma results from exposure of the cells defining said pathway to a cellular toxin.
36. The method of claim 35 wherein said toxin comprises ethanol.
37. The method of claim 29 wherein said damaged neural pathway results from a neuropathy of the cells defining said pathway.
38. The method of claim 37 wherein the etiology of said neuropathy is metabolic, infectious, toxic, autoimmune, nutritional, or ischemic.
39. The method of claim 38 wherein said neuropathy comprises Parkinson's disease, Huntington's chorea, amyotrophic lateral sclerosis, multiple sclerosis, or Alzheimer's disease.
40. The method of claim 37 wherein said neuropathy comprises axonal degeneration.
41. The method of claim 37 wherein said neuropathy comprises a demyelinating neuropathy.
42. The method of claim 29 wherein said damaged neural pathway results from a neoplastic lesion.

43. The method of claim 42 wherein said neoplastic lesion is caused by a neuroblastoma or a glioma.
44. The method of claim 29 wherein said morphogen stimulates cell adhesion molecule production in a cell defining said pathway.
45. The method of claim 44 wherein said cell adhesion molecule is a nerve cell adhesion molecule.
46. The method of claim 45 wherein nerve cell adhesion molecule is selected from the group consisting of N-CAM-120, N-CAM-140 and N-CAM-180.
47. The method of claim 29 or 46 wherein said morphogen comprises an amino acid sequence sharing at least 70% homology with one of the sequences selected from the group consisting of: OP-1, OP-2, CBMP2, Vgl(fx), Vgr(fx), DPP(fx), GDF-1(fx) and 60A(fx).
48. The method of claim 47 wherein said morphogen comprises an amino acid sequence sharing at least 80% homology with one of the sequences selected from the group consisting of: OP-1, OP-2, CBMP2, Vgl(fx), Vgr(fx), DPP(fx), GDF-1(fx), and 60A (fx).
49. The method of claim 48 wherein said morphogen comprises an amino acid sequence having greater than 60% amino acid identity with the sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1.)
50. The method of claim 49 wherein said morphogen comprises an amino acid sequence having greater than 65% amino acid identity with the sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1.)

51. The method of claim 50 wherein said morphogen comprises an amino acid sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1), including allelic and species variants thereof.
52. A method of maintaining a neural pathway in a mammal comprising:
administering said mammal an effective amount of an agent capable of stimulating production of an endogenons morphogen in a cell defining said pathway.
53. A composition for promoting regeneration of a neural pathway at a site of injury in a mammal, comprising:
a biocompatible, in vivo bioresorbable carrier suitable for maintaining a protein at a site in vivo, and
a morphogen, such that said morphogen, when dispersed in said carrier and provided to said site of injury, is capable of stimulating neural pathway regeneration at said site.
54. The composition of claim 53 wherein said carrier is structurally sufficient to assist direction of axonal growth.
55. The composition of claim 54 wherein said carrier comprises a polymeric material.
56. The composition of claim 54 wherein said carrier comprises laminin or collagen.

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57. A device for repairing a break in a neural pathway, the device comprising:

a biocompatible tubular casing comprising an exterior and an interior surface and defining a channel through which a neural process may regenerate,

said device having a shape and dimension sufficient to span a break in a neural pathway, and having openings adapted to receive the ends of a severed nerve, and

a morphogen disposed within the channel defined by said tubular casing and accessible to severed nerve ends defining a break in a neural pathway, such that said morphogen stimulates neural pathway regeneration when disposed in said channel and accessible to said nerve ends.

58. The device of claim 57 wherein said morphogen is disposed in said channel together with a biocompatible, bioresorbable carrier suitable for maintaining a protein at a site in vivo.
59. The device of claim 58 wherein said carrier comprises sufficient structure to assist direction of axonal growth within said channel.
60. The device of claim 57 wherein the outer surface of said casing is substantially impermeable.
61. The device of claim 58 wherein said carrier comprises a polymer.
62. The device of claim 58 wherein said carrier comprises laminin or collagen.

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63. A method for inducing the redifferentiation of transformed cells of neural origin, the method comprising the step of:
- contacting said transformed cells with a morphogen composition at a concentration and for a time sufficient to induce redifferentiation of said cells to a morphology characteristic of untransformed neuronal cells.
64. The method of claim 63 wherein said morphology characteristic of untransformed nerve cells includes formation of neurite outgrowths.
65. The method of claim 63 wherein said morphology characteristic of untransformed nerve cells includes cell aggregation and cell adhesion.
66. The method of claim 63 wherein said morphogen composition induces nerve cell adhesion molecule production in said cells.
67. The method of claim 63 wherein said induced nerve cell adhesion molecules include N-CAM-180, N-CAM-140 and N-CAM-120.
68. The method of claim 63 wherein said transformed cells comprise neuroblastoma cells.
69. A method for detecting a neuropathy in a mammal, the method comprising the step of:
- detecting fluctuations in the physiological concentration of a morphogen present in the serum or cerebrospinal fluid of said mammal, said fluctuations being indicative of an increase in neuronal cell death.

70. A method for detecting a neuropathy in a mammal, the method comprising the step of:
detecting fluctuations in the physiological concentration of a morphogen antibody titer present in the serum or cerebrospinal fluid of said mammal, said fluctuations being indicative of an increase in neuronal cell death.
71. The method of claim 69 or 70 wherein said neuropathy results from a neurodegenerative disease, nerve demyelination, myelin dysfunction, neuronal neoplasias, or nerve trauma.
72. A method of stimulating production of cell adhesion molecules in a tissue comprising the step of:
providing a morphogen to said tissue for a time and at a concentration sufficient to induce production of cell adhesion molecules in cells of said tissue.
73. The method of claim 72 wherein said cell adhesion molecules comprises nerve cell adhesion molecules.
74. The method of claim 73 wherein said cells comprise neurons.
75. The method of claim 69, 70 or 72 wherein said morphogen comprises an amino acid sequence sharing at least 70% homology with one of the sequences selected from the group consisting of: OP-1, OP-2, CBMP2, Vgl(fx), Vgr(fx), DPP(fx), GDF-1(fx) and 60A(fx).

76. The method of claim 75 wherein said morphogen comprises an amino acid sequence sharing at least 80% homology with one of the sequences selected from the group consisting of: OP-1, OP-2, CBMP2, Vgl(fx), Vgr(fx), DPP(fx), GDF-1(fx) and 60A (fx).
77. The method of claim 76 wherein said morphogen comprises an amino acid sequence having greater than 60% amino acid identity with the sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1.)
78. The method of claim 77 wherein said morphogen comprises an amino acid sequence having greater than 65% amino acid identity with the sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1.)
79. The method of claim 78 wherein said morphogen comprises an amino acid sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1), including allelic and species variants thereof.
80. A composition for enhancing survival of neuronal cells at risk of dying comprising a morphogen in association with a molecule capable of enhancing the transport of said morphogen across the blood-brain barrier.
81. The invention of claim 53 or 57 wherein said carrier comprises brain tissue derived extracellular matrix.

Abstract of the Disclosure

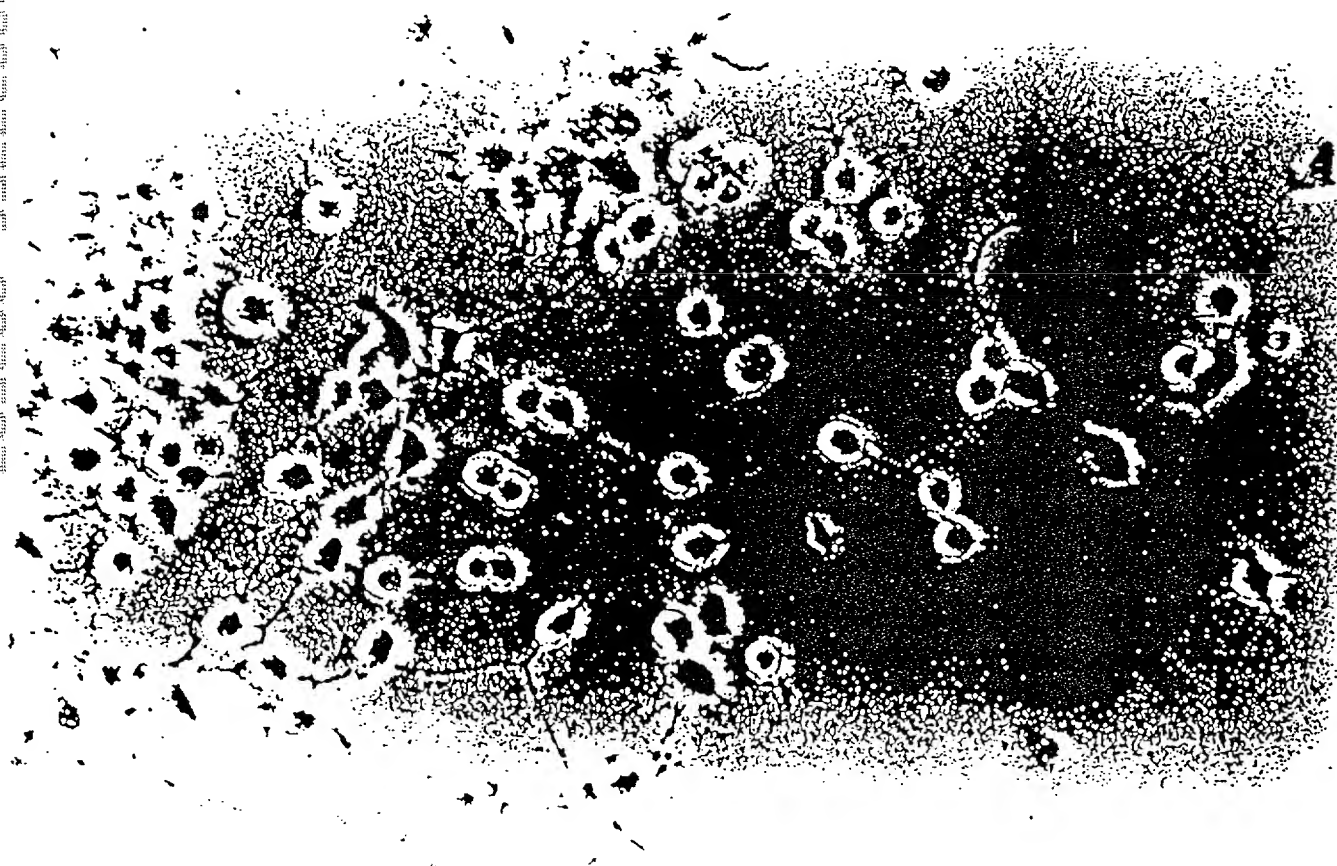
Disclosed are therapeutic treatment methods, compositions and devices for maintaining neural pathways in a mammal, including enhancing survival of neurons at risk of dying, inducing cellular repair of damaged neurons and neural pathways, and stimulating neurons to maintain their differentiated phenotype. In one embodiment, the invention provides means for stimulating CAM expression in neurons. The invention also provides means for evaluating the status of nerve tissue, including means for detecting and monitoring neuropathies in a mammal. The methods, devices and compositions include a morphogen or morphogen-stimulating agent provided to the mammal in a therapeutically effective concentration.

A



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B.



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FIG. 1 A-B

OP-1 Differentially Induces 180 and 140 kDa N-CAM

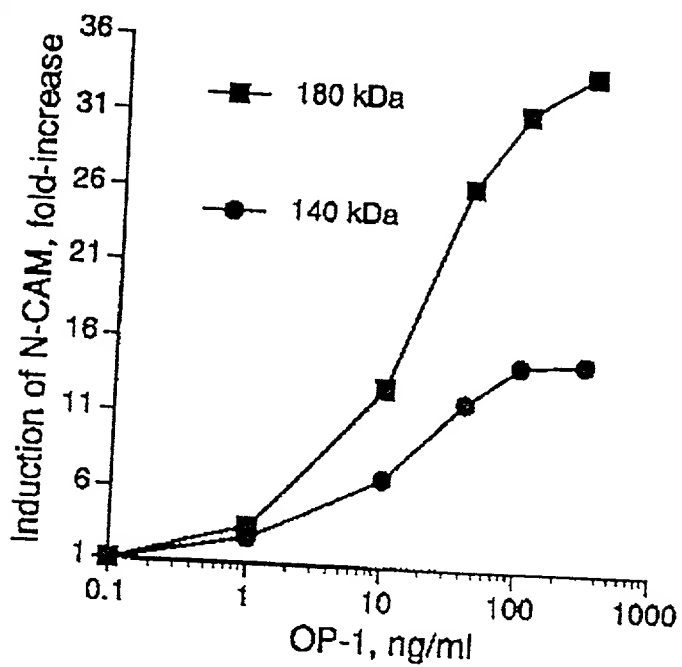


FIG. 2A

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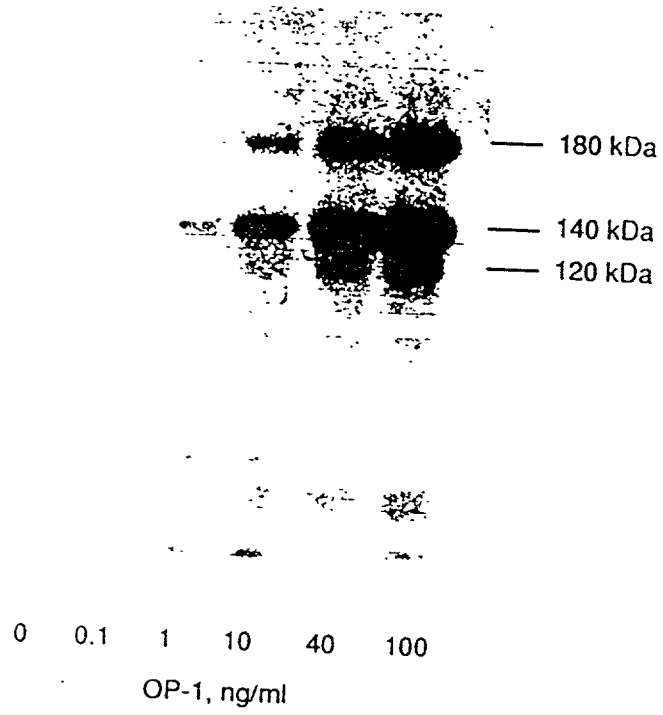


Fig 2B

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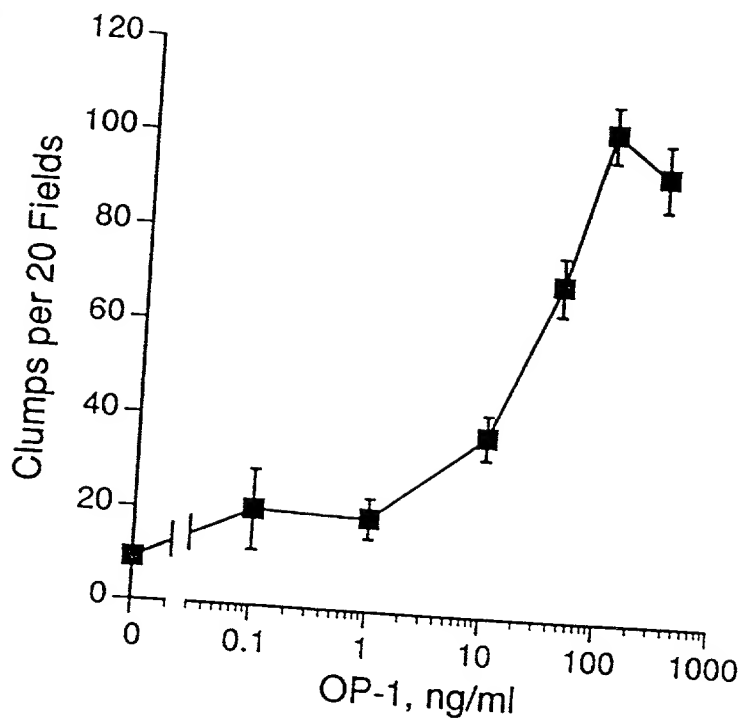


Fig. 3

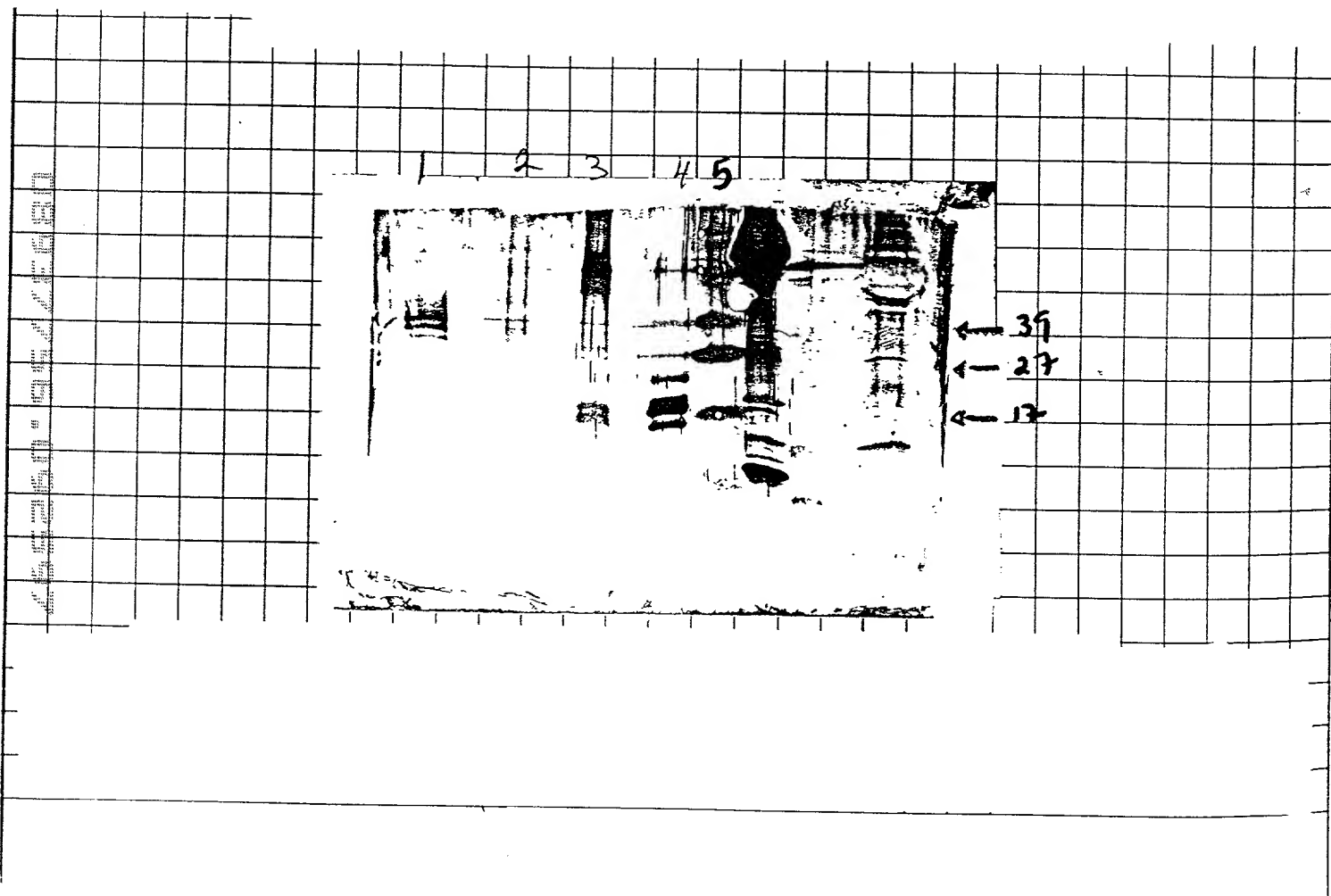


Figure 4